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# ANNALS OF BOTANY

EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

EMERITUS PROFESSOR OF PLANT PHYSIOLOGY, IMPERIAL  
COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

ASSISTED BY

A. J. EAMES, Ph.D.

PROFESSOR OF BOTANY, CORNELL UNIVERSITY  
ITHACA, N.Y., U.S.A.

AND OTHER BOTANISTS

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With nine Plates,  
two hundred and one Figures,  
and four Tables

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# Autopolyploid and Allopolyploid Watercress with the Description of a New Species

BY

H. W. HOWARD

(School of Agriculture, Cambridge)

AND

I. MANTON

(Botany Department, University of Manchester)

With Plate I and five Figures in the Text

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## INTRODUCTION

THE geographical distribution, history, morphology, and cytology of diploid, triploid, and wild tetraploid watercresses were described in some detail by Manton (1935). Later a short note by Howard and Manton (1940) reported that the wild tetraploid was an allotetraploid. The present paper is an amplification of the short statement of 1940 and the evidence for allopolyploidy is, for the first time, adequately documented. In the light of it, it is desirable, in the opinion of the taxonomists whom we have consulted, to rename the wild forms known previously as diploid, triploid, and tetraploid *Nasturtium officinale* R.Br. The new specific name of *N. uniseriatum* is proposed for the allotetraploid, the old name of *N. officinale* R.Br. (sensu stricto) being reserved for the diploid. The triploid, as previously shown, is the hybrid between them. Descriptions and diagnoses of these three forms are provided.

As explained in the preliminary note, the demonstration of allopolyploidy in the wild tetraploid involved firstly the production of an autotetraploid directly from the diploid by colchicine treatment. This tetraploid was found to differ markedly from the wild tetraploid both in appearance and behaviour and, when crossed with the wild tetraploid, it produced a sterile hybrid. Analysis of the chromosome pairing in meiosis then completed the evidence. Some additional evidence from the morphology of the plants and from the



breeding behaviour of the triploid is also available and is described in this paper.

#### PRODUCTION OF THE AUTOTETRAPLOID

The colchicine treatment consisted in placing drops of a 1 per cent. aqueous solution of the drug between the cotyledons of young diploid seedlings. Drops were given on successive days, one drop per day. The seedlings were treated when the shoot was only just visible between the expanded cotyledons. The advantage of this method of colchicine treatment is that root growth is not checked.

Sixteen seedlings were treated. Four received one drop, another 4 two drops, the third 4 three drops, and the remaining 4 four drops. The seedlings treated with one drop flowered first. Three of these were tetraploid and one diploid. The remaining 12 plants were not examined carefully, but it is known that they included one octoploid.

The tetraploid inflorescences were first recognized by measuring pollen-grain size. Diploid plants have pollen measuring  $7 \times 6$  arbitrary units (actual size  $22 \mu \times 19 \mu$ ) as compared with  $8 \times 7$  units (actual size  $24 \mu \times 21 \mu$ ) for the tetraploid. The initial diagnoses were confirmed by the reduced fertility of the autotetraploids and by their behaviour in crosses with the diploid controls. Finally, their progeny were examined cytologically a year later and found to be tetraploid; see Pl. I, Fig. 12.

When seeds from the tetraploid inflorescences were grown side by side with seeds from the diploid parent, the autotetraploid was found to resemble the diploid far more closely than it did the wild tetraploid. Thus though the leaves of the autotetraploid were somewhat thicker and darker in colour than those of the diploid and the plants were somewhat larger both as a whole and in all their parts, the delayed flowering and straggling habit previously noticed in the wild tetraploid (Manton, 1935, and p. 3 below) were not displayed and the fruits, in spite of their reduced fertility, resembled those of the diploid and not those of the wild tetraploid. Some of these features can be seen at a glance in Pl. I, Figs. 1, 4, and 6, and they will be referred to again in the section on morphology (p. 8 below).

#### HYBRIDIZATION EXPERIMENTS AND FERTILITY OF THE VARIOUS FORMS

Starting with single strains<sup>1</sup> of diploid, autotetraploid, and wild tetraploid, all possible cross and self-pollinations were attempted, with results indicated in Tables I and II.

TABLE I  
*Results of Self-Pollinations, Cambridge, 1939*

Plants.	Seeds per fruit.	Average seed weight (mg.).
Diploid	26 large good	0.235
Wild tetraploid	29     "	0.208
Autotetraploid	11     "	0.324

<sup>1</sup> Diploid from Zürich; wild tetraploid from Wareham.

Table I shows the effect of selfing, and the reduced number but larger size of the seeds in the autotetraploid are at once apparent. Their large size is perhaps in part the expression of the extra food made available by their reduced number. The number of seeds per fruit is less than half that in the diploid, and in this the autotetraploid is similar to autotetraploid *Brassica oleracea* (Howard, 1939) and autotetraploid *Lycopersicum esculentum* (Sansome, 1933) which have seed fertilities of 30 and 20 per cent. of their diploids respectively.

Of the six possible types of cross pollination listed in Table II, all produced big fruits, but in only two cases did these contain viable seeds. The small size of the seeds in these two cases is noteworthy; exactly comparable results were

TABLE II  
*Results of Cross Pollinations, Cambridge, 1939*

Female parent.	Male parent.	Seeds per fruit.	Average seed weight (mg.).
Diploid	Wild tetraploid	14 large empty	—
Diploid	Autotetraploid	9 small empty	—
Wild tetraploid	Diploid	9 small good	0.075
Wild tetraploid	Autotetraploid	12 large empty	—
Autotetraploid	Diploid	22 small empty	—
Autotetraploid	Wild tetraploid	15 small good	0.124

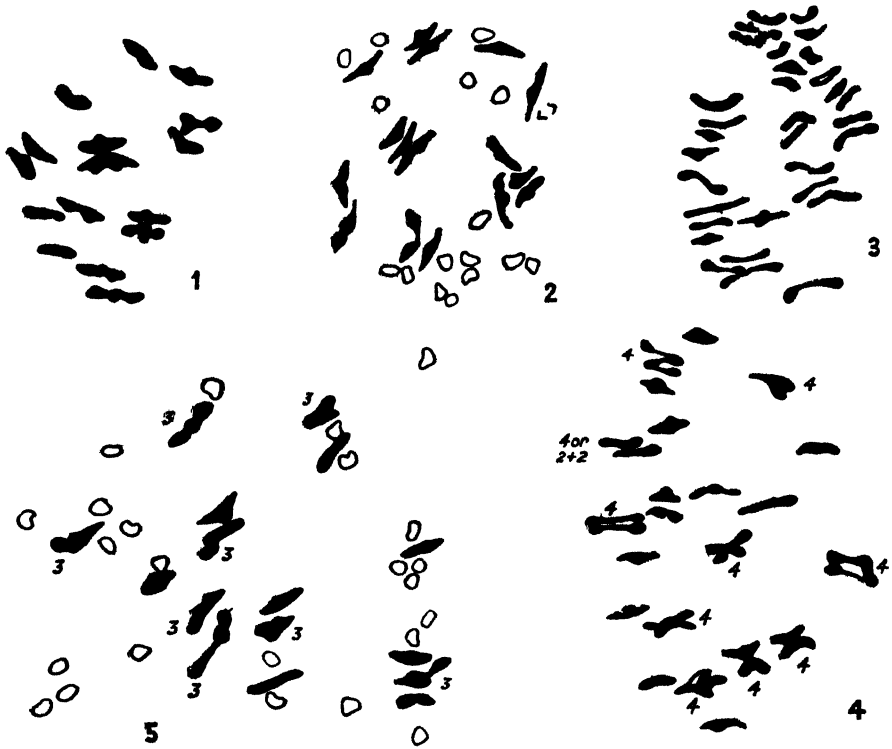
obtained in crosses between autotetraploid and diploid *Brassica oleracea* (Howard, 1939) and in crosses between amphidiploid *Brassica chinensis-carinata* and its two parent species (Howard, 1942). The autotriploid has proved impossible to obtain with the strains used. The hybrid triploid (wild tetraploid  $\times$  diploid) was also obtained by Manton (1935).

The 'hybrid tetraploid', as the cross between autotetraploid and wild tetraploid may conveniently be called, is of particular importance in unravelling the cytological nature of the wild tetraploid. A photograph of it is given in Pl. I, Fig. 3, reduced to the same scale as that of the diploid and wild tetraploid shown in Pl. I, Figs. 1 and 2 respectively. The plants were vigorous and large and in habit were intermediate between the wild tetraploid and the autotetraploid, having some of the straggling tendency of the former (Pl. I, Fig. 2), though in a lesser degree. The seed fertility was very low indeed, as may be seen from Table III.

TABLE III  
*Fertilities of the various Forms of Watercress at Manchester, 1940*

Plant.	Percentage good pollen.	Percentage of ovules		
		Undeveloped.	Aborted seeds.	Good seeds
Wild tetraploid . . .	99	16	4	80
Diploid . . .	97	20	5	75
Autotetraploid . . .	91	58	28	16
Hybrid tetraploid . . .	53	74	23	3
Hybrid triploid . . .	20	73	20	7

It is obvious from Table III that the low percentage of good seeds set by the last three plants listed is due to the sum of two processes. Many of the ovules formed develop no further—a proportion of these presumably contained non-viable megaspores (cf. the percentage of bad pollen). Of those ovules which start to develop further and therefore presumably were fertilized, a



TEXT-FIGS. 1-5. Analysis of chromosome pairing in the photographed cells of the five types of plant examined. Text-fig. 1. The diploid cell of Pl. I, Fig. 8, showing 16 pairs. Text-fig. 2. The triploid cell of Pl. I, Fig. 9, showing 16 pairs in black and 16 univalents in outline. Text-fig. 3. The tetraploid cell of the wild tetraploid (*N. uniseriatum* sp. nov.) of Pl. I, Fig. 14, showing 32 pairs. Text-fig. 4. Cell of the autotetraploid *N. officinale* R.Br. from Pl. I, Fig. 13, quadrivalents labelled, pairs unlabelled. Text-fig. 5. Cell of the hybrid between wild and autotetraploids shown in Pl. I, Fig. 10; univalents in outline, pairs in black and trivalents in black with labels. Further description of this in text and Table IV. ( $\times 2,000$ .)

large proportion fail to reach maturity—some of these presumably contained non-viable zygotes. The low seed fertility of the hybrid tetraploid is in itself strong presumptive evidence for the wild tetraploid being an allotetraploid.

#### CYTOLOGICAL INVESTIGATION OF MEIOSIS

Conclusive evidence for allopolyploidy in the wild tetraploid is provided by the analysis of chromosome pairing at meiosis in the five types of plant of Table III. Three of these, namely the diploid, wild tetraploid, and hybrid

triploid, were examined with some care in 1935. Special search had been made for the presence of multivalents but they had not been found. Instead, the wild tetraploid appeared to form 32 pairs at meiosis in contrast to the 16 pairs formed by the diploid. The triploid had 16 paired and 16 unpaired chromosomes in its pollen mother-cells (Manton, 1935). Since the chromosomes of all the watercresses are very small indeed (see for example Pl. I, Fig. 15), it was thought by Manton (1935) that the absence of multivalents from the wild polyploids might be due to the small size of chromosomes and correspondingly low chiasma frequencies. It was also possible that they might, in spite of efforts, have been overlooked.

Using the newer methods subsequently worked out for *Biscutella* (Manton, 1937) by which the contents of the nuclei can be spread out and artificially enlarged in a permanent aceto-carmin squash, the three wild-type plants have been re-examined to the extent necessary to confirm the previous account. The results appear in Pl. I, Figs. 8, 9, and 14 and with explanatory diagrams in Text-figs. 1-3. Pl. I, Fig. 6 and Text-fig. 1 shows the 16 pairs of the diploid. Pl. I, Fig. 9 and Text-fig. 2 shows the 16 pairs and 16 univalents characteristic of the triploid,<sup>1</sup> while Pl. I, Fig. 14 and Text-fig. 3 shows the 32 pairs of the wild tetraploid. The absence of multivalents from the wild-type plants is thus confirmed.

In contrast with this the presence of quadrivalents in the autotetraploid is unmistakable. They are seen with great clarity in the photograph of Pl. I, Fig. 13 in attitudes which could not easily be imitated by casual juxtaposition of separate bivalents. As is shown in Text-fig. 4, the nucleus contains 9-10 quadrivalents. The presence of quadrivalents in the autotetraploid thus suggests strongly that their absence from the wild tetraploid is not due to a low chiasma frequency but that it is a question of homology.

Chromosome pairing in the hybrid tetraploid is shown in Pl. I, Fig. 10 and in the diagram of Text-fig. 5. It is as critical to the analysis of the wild tetraploid as is pairing in the hybrid triploid. Quadrivalents are absent, but trivalents, bivalents, and univalents occur. The high frequency of univalents can also be seen in the anaphase shown as Pl. I, Fig. 11; they are seen lagging on the spindle and dividing. Table IV summarizes the analysis of six pollen mother-cells of the hybrid tetraploid, and it is important to note that the sum of bivalents and trivalents always equals the monoploid chromosome number of 16.

TABLE IV

*Analysis of Chromosome Pairing in 6 Pollen Mother-Cells of the Hybrid Tetraploid*

Type of nucleus.	Number of cells observed.
7 trivalents + 9 bivalents + 25 univalents	1
8 trivalents + 8 bivalents + 24 univalents	3
9 trivalents + 7 bivalents + 23 univalents	0
10 trivalents + 6 bivalents + 22 univalents	1
11 trivalents + 5 bivalents + 21 univalents	1

<sup>1</sup> The triploids used on this occasion were not wild but had been synthesized from the cross between wild tetraploid ♀ × diploid.

There is only one interpretation to fit all the cytological results. The trivalents and bivalents of the hybrid tetraploid and the bivalents of the hybrid triploid must be watercress chromosomes strictly homologous with those of the diploid, but they only represent half the chromosome complement of the wild tetraploid. This may be expressed as follows. The chromosome constitution of diploid watercress can be represented as  $AA$ , where  $A$  = the 16 chromosomes of the gametic set, and the wild tetraploid as  $AABB$ , where  $B$  = the 16 chromosomes of the gametic set of some other at present unidentified cruciferous plant. Thus the autotetraploid will be  $AAAA$ , the hybrid tetraploid  $AAAB$ , and the hybrid triploid  $AAB$ . This interpretation clearly shows why the hybrid tetraploid forms trivalents and bivalents but not quadrivalents, and why the hybrid triploid forms bivalents and univalents but not trivalents.

#### ADDITIONAL OBSERVATIONS ON BREEDING IN THE TRIPLOID HYBRID

The further breeding behaviour of the triploid is of some interest as an indication of additional evolutionary possibilities in the wild plants. It suggests a means by which structural hybrids or new forms with aneuploid chromosome numbers may be produced.

As is well known, the meiotic process in triploids will generally effect a distribution of chromosomes to the spores in numbers ranging from  $n$  to  $2n$  with approximately binomial frequency. Most of these spores will be non-viable, and the progeny of a triploid will therefore, as a rule (see Sansome and Philp, 1939, p. 231), tend to contain only the regular polyploid chromosome numbers, chiefly diploid and tetraploid, or close approximations thereto.

Evidence published earlier (Manton, 1935) indicated that wild triploid watercress does not behave in this manner. These observations have since been repeated and extended, using triploids of known parentage. The evidence is of two kinds—firstly, from triploids left alone to self-pollinate, and secondly, from triploids back-crossed to both diploid and tetraploid parents. The chromosome numbers of the progeny were determined in each case.

Table V summarizes the chromosome numbers of 11 plants obtained by self-pollination of hybrid triploids, the upper row of 6 plants being those discussed by Manton (1935). The very wide range of chromosome numbers is noteworthy. The regular polyploids are absent but they would probably be obtained if larger families were raised. The most striking feature is, however, the high degree of tolerance for unbalanced cytological types.

TABLE V

#### *Chromosome Numbers of the Progeny of Self-Pollinated Triploids ( $3n = 48$ )*

Source.	Number of plants with chromosome number of:																	
	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Wild triploids . . . . .	1	—	—	—	—	—	—	1	—	—	1	2	—	—	—	—	—	1
Synthesized triploids . . . . .	—	—	—	—	—	—	1	—	1	—	—	—	—	1	—	—	1	—
Total (11 plants) . . . . .	1	—	—	—	—	—	1	1	1	—	1	2	—	1	—	—	1	2

For wild triploids see Manton, 1935. The synthesized triploids were from the cross wild tetraploid female  $\times$  diploid.

Table VI summarizes the chromosome numbers of 14 plants obtained from 4 families of the back-cross between triploid female  $\times$  diploid. The reciprocal cross is represented by one family of two plants. Two additional plants obtained from the cross triploid female  $\times$  wild tetraploid had chromosome numbers of 56 and 59; they are not included in Table VI but are utilized in Table VII.

TABLE VI

*Chromosome Numbers of Plants from the Cross Triploid Female  $\times$  Diploid*  
( $2n = 32$ ,  $3n = 48$ )

Source.	Number of plants with chromosome number of:															
	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47 48
$3n_{\text{f}} \times 2n_{\text{m}}$	—	—	—	—	1	4	—	1	2	2	2	1	—	1	—	—
$2n_{\text{f}} \times 3n_{\text{m}}$	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—
Total (16 plants)	—	—	—	—	1	5	1	1	2	2	2	1	—	1	—	—

The triploids were from the cross wild tetraploid female  $\times$  diploid.

Table VII shows the chromosome numbers of the gametes of triploid origin involved in the production of the 16 plants listed in Table VI and of the two additional plants mentioned in the last paragraph. Each gamete produced by the triploid must contain the complete set of 16 chromosomes (the *A* set) of diploid watercress, but in addition a highly variable number of chromosomes from the *B* set. The great tolerance to unbalance is again the striking feature, as in the case of the progeny from the triploid when selfed. A similar tolerance to unbalance has been found in the progeny of auto-triploid *Pyrus Malus* (Moffett, 1931).

TABLE VII

*Numbers of Chromosomes above the Haploid Number of 16 found in functional Gametes from triploid Watercress*

Cross.	Numbers of supernumerary chromosomes:															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
$3n_{\text{f}} \times 2n$	—	—	—	1	4	—	1	2	2	2	1	—	1	—	—	—
$3n_{\text{m}} \times 2n$	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—
$3n_{\text{f}} \times \text{wild } 4n$	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—	—
Total (18 plants)	—	—	—	1	5	1	1	3	2	2	2	—	1	—	—	—

It may be said in passing that all the plants obtained from the triploid resembled normal types of *Nasturtium officinale* sufficiently closely to be classed as that species, though most were depauperate and some misshapen. Their low seed fertility would have caused almost all of them to be diagnosed as triploids if found wild. Some of them, however, flowered profusely, and the fertility of 10 plants was determined. The percentages of good seed relative to ovules ranged from 2 to 13 per cent. and were thus of the same order as that found in their triploid parent (see Pl. I, Fig. 7, and Table III). One plant with 39 chromosomes (see Pl. I, Fig. 15), from the wild triploid

selfed, was more fertile and had 30 per cent. good seeds (see Pl. I, Fig. 7, top right). Such a plant could be of importance from an evolutionary point of view.

The evolutionary consequences of a breeding behaviour such as is found in triploid watercress were touched on in a previous paper (Manton, 1935). It is obvious that a tolerance of unbalance on the scale shown could lead to the production of new true-breeding forms of more than one kind. If any of the supernumerary *B* chromosomes were to obtain partners as a result of continued self-pollination, they could be permanently retained in the gametic complement and a departure from the basic haploid number of 16 result if back-crossing to diploid and tetraploid were prevented. Even in the absence of such isolation an important influence could still be exerted by the triploid if translocations or segmental interchange were to take place between chromosomes of the *A* and *B* sets. In this case the chromosome number would remain unchanged, but a structural hybrid, involving recombination of chromosomes or parts of chromosomes of several taxonomic species, would result. How far these processes will actually be carried in the wild watercress populations is of course unknown, but further examination of them from this point of view would not be unprofitable.

#### MORPHOLOGY OF THE WILD FORMS

In the vegetative condition all forms of watercress are so similar that very close scrutiny is necessary to distinguish them, though they can be separated easily when fruiting. Since the autotetraploid and hybrid tetraploid are of experimental origin and do not occur wild, further description of them may be omitted. The three remaining types—diploid, wild tetraploid, and their triploid hybrid—are, however, important and widespread elements in the European flora (Manton, 1935), and a brief summary of the various diagnostic characters by which they may be recognized is therefore given.

(a) *Growth habit*. It is doubtful whether this character can be used with wild plants owing to their great plasticity under different environmental conditions. When cultivated under identical conditions the wild tetraploid flowers later than the diploid; the difference was a fortnight at Manchester in 1940. The wild tetraploid also tends to flower less freely than the diploid, and it forms an abundance of barren shoots which conduce to ease of perennation. The diploid, on the other hand, will tend to behave as an annual unless care is taken to stimulate branching from the base when the fruits are ripening. The straggling habit of the wild tetraploid is also evident even in quite young seedlings as may be seen in Pl. I, Fig. 4.

(b) *Stomatal index*. This is the only character by which wild material devoid of fruits can be identified with certainty without a chromosome count. We are indebted to Dr. J. M. Rowson of the Pharmacy Department, Sunderland Technical College, and formerly of Manchester University, for working out the details for us and for supplying the following account:

'The stomatal index of a leaf was defined by Salisbury (1927) as the ratio of the number of stomata to the total number of epidermal initials, according to the equation: Stomatal Index =  $\frac{S}{E+S} \times 100$ , where  $E$  denotes the number of epidermal

cells in unit area, and  $S$  the number of stomata in the same area. It has been shown that, while stomatal number per unit area is very variable within a species, stomatal index is highly constant and is independent of age, position of leaf surface (excluding extreme apex, margin or midrib), and geographical habitat. Stomatal indices have been used successfully in pharmacognosy to distinguish species of economic importance in the following genera: *Cassia* (Rowson, 1943), *Erythroxylum* and *Atropa* (Rowson, 1943a), and *Datura*, *Hyoscyamus*, and *Digitalis* (Rowson, in the press).

'In determining the stomatal index, strips of epidermis are removed from the upper and lower surfaces of five representative leaves. Using a squared eyepiece micrometer disk and suitable combination of lenses to give a count of 20–60 cells per field, counts of the number of stomata and epidermal cells are made in consecutive fields (avoiding the apex, base, margin, and midrib) until about 400 cells have been enumerated. The stomatal index is then calculated from the above equation.

'For the three wild forms of watercress observations were made on wild plants from three different localities in each case. Agreement between plants of different origin was close and only affected the size of the decimal fraction. The stomatal indices characteristic of the different types are summarized in Table VIII.'

TABLE VIII  
*Stomatal Indices of Watercress*

	Material.		Lower surface.	Upper surface.
2n	Dr. Howard, Cherry Hinton	15/11/1944	17.0	15.0
	„ Barton	18/6/1945	18.2	15.1
	„ King's College	18/6/1945	18.0	14.9
	AVERAGE OF SAMPLES		17.7	15.0
3n	Dr. Manton, Adlington	16/11/1944	15.1	13.0
	Dr. Howard, Cultivated, pot U*	23/6/1945	15.0	14.7
	„ Cultivated, pot AA*	24/6/1945	15.2	14.3
	AVERAGE OF SAMPLES		15.1	14.0
4n	Dr. Howard, Sheep's Green	15/11/1944	11.8	11.5
	Dr. Manton, Northenden	18/6/1945	11.2	10.3
	Dr. Howard, Cultivated, pot Do <sub>2</sub> *	23/6/1945	10.6	10.7
	AVERAGE OF SAMPLES		11.2	10.8

\* Pot U originally from Balerno, Scotland; pot AA from Lindores, Scotland; pot Do<sub>2</sub> from West Moors, Dorset; all grown in Cambridge.

(c) *Fruit shape*. This is the best taxonomic character by means of which all forms of watercress can at once be distinguished. Photographs were published in Manton (1935), but they are also given here because of their importance to the field botanist. The fruits of the diploid (Pl. I, Fig. 6, bottom) are the only ones which correspond to the usual description of *N. officinale* R.Br. The average range in length of the diploid fruits is 15–18 mm.; they



are borne on a stout pedicel 8–12 mm. long, and the seeds are very distinctly arranged in two rows (biseriate). The fruits of the wild tetraploid tend to be slightly longer, the average range in length being 18–22 mm., borne on a pedicel 11–15 mm. long, but both the fruit and the pedicel are thinner than in the diploid and the seeds are in only one row (uniseriate), see Pl. I, Fig. 6, top. This uniseriate arrangement of seeds, as was pointed out by Manton (1935), is a taxonomic character not of *Nasturtium* but of the related genus *Cardamine*. It confirms the cytological evidence that the wild tetraploid watercress is an allotetraploid and not an autotetraploid. The fruits of the triploid are conspicuous by their deformed or aborted appearance (see Pl. I, Fig. 7, left), the average number of seeds per fruit being less than one.

(d) *Seed character*. The markings on the testa provide a very distinctive feature for separating diploid and wild tetraploid. The distinction is clearly shown in Pl. I, Fig. 5. The testa of the diploid (Fig. 5, bottom) has a coarse areolation which is enhanced in the autotetraploid (Fig. 5, middle). In the wild tetraploid (Fig. 5, top) the areolation is much finer. The triploid seed is intermediate and the seeds are also smaller and less well filled.

#### SYSTEMATICS AND NOMENCLATURE

The name *Nasturtium officinale* was first used by Robert Brown in 1812 as a substitute for the Linnean name *Sisymbrium Nasturtium* (-*aquaticum*), the epithet being changed to avoid tautonymy. Brown did not cite any specimen for his new name, and thus the type specimen is still the one described by Linnaeus. Linnaeus' first treatment of watercress was in his 'Hortus Cliffortianus' of 1737 in which the description is 'Sisymbrium foliis pinnatis, foliolis subcordatis'. However, in his 'Species Plantarum' of 1753 (the first work in which he used the binomial nomenclature) Linnaeus named the species *Sisymbrium Nasturtium-aquaticum* and gives the following description: 'Sisymbrium siliquis declinatis, foliis pinnatis: foliolis subcordatis.' The 1753 description thus differs from the 1737 in that 'siliquis declinatis' has been added. The specimens in the Clifford Herbarium and in the Linnean Herbarium have been examined. The single sheet in the Linnean Herbarium is certainly of the diploid and the single sheet in the Clifford Herbarium almost certainly of the tetraploid. As is pointed out above, the 'Species Plantarum' definition is a new one and one can therefore cite the Linnean Herbarium specimen (i.e. the diploid) as the type for *Nasturtium officinale*. This also has the additional advantage that the diploid is the only wild form which fully corresponds to the usual diagnostic description of *Nasturtium officinale* R.Br. The diploid is also the most widespread in geographical range (Manton, 1935).

The triploid is equally certainly the hybrid between the wild tetraploid and *N. officinale* sensu stricto. Since its structure and behaviour is that customary in hybrids it needs no further designation.

The wild tetraploid is, however, now shown to be an allotetraploid with a

morphology which does not agree with either the generic or specific description of *N. officinale* R.Br. and which also forms a sterile hybrid when crossed with that species. This behaviour would have led inevitably to its separation from *N. officinale* as a new species if it had been found originally by a taxonomist. When the cytology was first investigated the question of nomenclature was left aside pending further evidence on the type of polyploidy involved. This evidence is now to hand and the question can no longer be ignored.

It is unfortunate that search has not yet revealed the identity of the other species involved in the original production of the allotetraploid. The genus *Nasturtium* in its modern sense (von Hayek, 1911; and others) is regarded as monotypic and its nearest relations are *Roripa* (previously included in *Nasturtium*) and *Cardamine*. Attempts at hybridizing *Roripa sylvestris* with both diploid and tetraploid watercress have failed completely, and it seems improbable that this genus is involved since it differs in many characters from watercress. The morphology of the fruits of wild tetraploid watercress points fairly clearly to the genus *Cardamine*, with particular likelihood of the species being a small white-flowered member of the section *Eu-cardamine*. The only species of this section at present known which has the appropriate chromosome number ( $n = 16$ ) is *C. flexuosa* With. (Manton, 1932). Repeated efforts by both authors to cross this species with watercress have, however, also failed. It is also important to note that *Nasturtium siifolium* and *microphyllum* of Reichenbach and *Nasturtium parvifolium* of Petermann all appear to be diploids and are thus not prior names for the tetraploid.

Under these circumstances it seems desirable to choose a name for the wild tetraploid which will express no more than the proved facts of structure and affinity. We therefore propose to call the wild tetraploid *Nasturtium uniseriatum* sp. nov. The tetraploid is thus, pending further information, retained within the genus to which its genetic relationship is proved.

#### DESCRIPTIONS OF THE TWO SPECIES AND OF THEIR HYBRID

*Nasturtium officinale* R.Br. sensu stricto. Perennial aquatic herb with glabrous pinnate leaves. Stem rooting at the nodes. Leaflets 3–6 pairs. Racemes short, flowers small with white petals which are twice as long as the sepals. Fruit with the double row of seeds very distinct. Seeds compressed, suborbicular with the testa having about 25 large depressions on each side. Mean stomatal index for lower epidermis of leaf 17.7. Chromosome number  $2n = 32$ .

*Nasturtium uniseriatum* sp. nov. Differs from the former in having longer and narrower fruits in which the seeds are arranged in a single row. Also the testa of the seeds has about 100 small depressions in each side. Stomatal index for the lower epidermis of leaf 11.2. Chromosome number  $2n = 64$ .

*Nasturtium uniseriatum*  $\times$  *officinale*. This hybrid may be recognized by its very short fruits which contain less than one good seed per fruit. Stomatal index of lower epidermis of leaf 15.1. Chromosome number  $2n = 48$ .

*Nasturtium officinale* sensu stricto. Herba aquatica, perennis, radicibus ex nodis emergentibus. Folia pinnata, glabra, foliolis 7-13. Racemi breves. Flores parvi, petalis albis sepalis bis longioribus. Semina biseriata, compressa, suborbicularia testis utrinque alveolis magnis circa 25 instructis. Chromosomata  $2n = 32$ .

*Nasturtium uniseriatum* Howard et Manton, sp. nov. A *N. officinale* fructibus longioribus angustioribusque, seminibus uniseriatis, testis utrinque alveolis parvis circa 100 instructis praecipue distinguitur. Chromosomata  $2n = 64$ . Typus; 'near Manchester', Manton, in Herb. Kew.

*Nasturtium uniseriatum*  $\times$  *N. officinale*. Hybrida, a *N. uniseriato* fructibus brevissimis circiter minus quam semen unum per fructum tenentibus distincta. Chromosomata  $2n = 48$ .

#### SUMMARY

1. By means of an autotetraploid produced from diploid watercress (*Nasturtium officinale* R.Br.) by colchicine treatment, the wild tetraploid watercress previously described by Manton (1935) has been shown to be an allotetraploid.

2. By analysis of chromosome pairing at meiosis in diploid, autotetraploid, wild tetraploid, and two hybrid forms of watercress, it has been shown that half the chromosomes of the wild tetraploid are homologous with the chromosomes of diploid *N. officinale* R.Br. The identity of the other half of the genome of the wild tetraploid is uncertain, but it is suspected to be a species of Cardamine.

3. The name *Nasturtium uniseriatum* is proposed for the wild tetraploid. Diagnoses of this new species and the other two wild forms of watercress are given.

4. The breeding behaviour of the wild triploid (*N. uniseriatum*  $\times$  *officinale*) is described. Its possible evolutionary significance is discussed.

Our thanks are due, among others, to Dr. L. C. Luckwill of Aberdeen for the seed weights, to Dr. J. M. Rowson of Sunderland for his contribution on stomatal index, to Dr. W. B. Turrill of Kew and Mr. J. E. Dandy of the British Museum for advice on systematics and nomenclature, and to Mr. A. E. Watkins of Cambridge who was responsible for reopening of the work after a lapse of several years.

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## DESCRIPTION OF PLATE I

Illustrating Dr. W. H. Howard and Dr. I. Manton's paper on 'Autopolyploid and Allopolyploid Watercress with the Description of a new Species'.

Fig. 1. Single plants of diploid (left) and autotetraploid (right) *N. officinale* R.Br., 4 months old, July, 1940.  $\frac{1}{4}$ th nat. size.

Fig. 2. Single plant of wild tetraploid watercress (*N. uniseriatum*), 3 months old, June, 1940.  $\frac{1}{4}$ th nat. size.

Fig. 3. Single plant of hybrid between wild tetraploid and autotetraploid watercress, 4 months old, July, 1940.  $\frac{1}{4}$ th nat. size.

Fig. 4. Pans of seedlings of diploid (left), wild tetraploid (centre), and autotetraploid (right). Note the early manifestation of the spreading habit of the wild tetraploid and close resemblance of autotetraploid and diploid.  $\frac{1}{10}$ th nat. size.

Fig. 5. Seeds of diploid *N. officinale* (bottom), autotetraploid *N. officinale*, and wild tetraploid = *N. uniseriatum* (top). ( $\times 14$ .)

Fig. 6. Fruits of diploid (bottom), autotetraploid (middle), and wild tetraploid (top). ( $\times 2$ .)

Fig. 7. Fruits of triploid hybrid between diploid *N. officinale* and wild tetraploid watercress (*N. uniseriatum*) on the left, and two of its progeny on the right; for description see text, p. 9. ( $\times 2$ .)

Fig. 8. Pollen mother-cell of diploid *N. officinale*,  $n = 16$ . Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 9. Pollen mother-cell of triploid watercress, showing 16 pairs and 16 univalents (cf. Text-fig. 2). Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 10. Pollen mother-cell of hybrid tetraploid (auto- $4n \times$  wild  $4n$ ), showing pairs, trivalents, and univalents (cf. Text-fig. 5 and Table IV). Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 11. The same plant as Fig. 10, showing behaviour of unpaired chromosomes at anaphase of the first meiotic division; they lag and divide. Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 12. Tapetal nucleus of autotetraploid *N. officinale* in late prophase, showing 64 chromosomes. Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 13. Pollen mother-cell of autotetraploid *N. officinale*, showing pairs and quadrivalents (cf. Text-fig. 4). Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 14. Pollen-mother cell of wild tetraploid (= *N. uniseriatum*), showing absence of quadrivalents (cf. Text-fig. 3). Permanent acetocarmine. ( $\times 1,500$ .)

Fig. 15. Somatic chromosomes from one of the progeny of the triploid (see text p. 7), showing 39 chromosomes. Section of a root stained in gentian violet. ( $\times 2,000$ .)







2



3



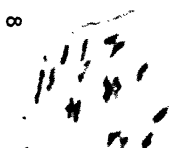
11



15



4



8



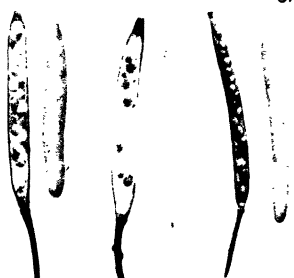
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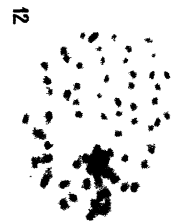
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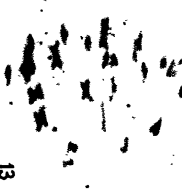
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12



13



14





# **A Study of Nut Grass (*Cyperus rotundus* L.) in the Cotton Soil of the Gezira**

## **II. The Perpetuation of the Plant by means of Seed**

BY

F. W. ANDREWS

*(Research Division, Dept. of Agriculture and Forests, Sudan Government)*

**With four Figures in the Text**

**I**N Part I of these studies an account was given of the life-history of the tuber of this plant (Andrews, 1940). It was shown that under the Sudan Gezira climatic conditions the tubers would die rapidly if their root systems were severed during the dry season. A method of control of this weed, developed from a knowledge of this fact, was also described.

In this paper it is proposed to describe the formation and dispersal of the fruit of nut grass and the part played by this fruit in the infestation of the Gezira irrigated cotton land.

The Gezira cotton soil (Joseph, 1925) is a heavy clay, strongly alkaline (pH up to 9.4), of low permeability to water and of low content of nitrogen and humus.

### **INTRODUCTION**

Little evidence is available that the seed of nut grass is to be considered an important means of propagation of this weed. Ranade and Burns (1925) working in India record that under experiment only 1.5 per cent. germination was obtained out of 22,086 seeds sown, and that many of the seedlings withered. They conclude that in the field even in the rains the germination percentage is low and many seedlings die, but on account of the enormous quantity of seed produced, the numbers of survivors may necessitate eradication of the weed before seed is set. Smith and Fick (1937) working in America state that the junior author was unable to find viable seed.

### **DISTRIBUTION AND DESCRIPTION OF NUT GRASS**

This plant is widely distributed over the Gezira irrigated cotton land where in localized areas it is the dominant weed. In low-lying areas, subject to flooding during the rains, the plant grows thickly and the flowering stem reaches a height of 2½ ft. On the bulk of the irrigated land the average height of the flowering stem is about 15 in. In thickly infested areas the weeding of the land immediately before the sowing of the cotton crop becomes arduous.

When nut grass is growing thickly among the crop, cotton plants are yellow and stunted and are obviously suffering from nutrient deficiency.

The plant is also one of the major weeds infesting the irrigation canals, where it lines both the banks and the water's edge, and will, if the water is shallow enough, also infest the canal bed.

A general description of the nut grass plant in the cotton soil of the Gezira has been given by Andrews (1940). The flowers of this plant are supported on a solid, smooth, triquetrous stem leafless except at the base. The inflorescence is umbellate and varies in the Gezira from 5 to 10 cm. in diameter, with 3 to 4 bracts overtopping the umbel. The number of spikelets varies from 15 to 35. The spikelets are loosely spicate, ferruginous-red to chestnut red in colour, average 15 mm. in length, and contain 8 to 20 flowers. The fruit is a trigonous obovoid dark brown nut averaging 1.2 mm. in length. The seed is obovoid with a thin testa. The fruit is indehiscent and is shed as such.

#### THE PRODUCTION OF FLOWERS IN RELATION TO WEATHER CONDITIONS

Table I shows the monthly averages for maximum and minimum temperatures, relative humidity, and rainfall at the Gezira Research Farm where this work was carried out.

TABLE I

*Monthly Meteorological Averages, Gezira Research Farm, Wad Medani 1919-42*

Month.	Average max. temp. ° C.	Average min. temp. ° C.	Relative hum. 8 a.m.	Average rain- fall (mm.)
January	33.9	14.2	36	—
February	35.4	15.1	26	—
March	38.4	17.7	20	—
April	41.1	20.9	17	3.3
May	41.0	23.7	32	11.0
June	39.7	24.4	46	33.3
July	35.6	22.5	68	132.2
August	33.5	22.0	76	146.1
September	35.8	22.0	68	55.5
October	38.5	21.9	48	13.3
November	36.8	18.2	33	1.1
December	34.5	15.2	37	—
			TOTAL	395.8

It will be noted that most of the rain falls within a space of 3 months. Because of this restricted rainfall, *Cyperus rotundus* L. in common with all other weeds on non-irrigated land has to produce its flowers and fruits during the rainy season. From the beginning of November the aerial portion of the plant dies down and the tubers lie dormant in the soil until the return of the heavier rains in July when fresh aerial growth is produced. On non-irrigated land fruiting therefore occurs annually during the period July to October and the land becomes sown with dropped fruits.

In a study of the perpetuation of this weed it was of importance to determine if during the irrigation of a cotton crop from October to April, nut-grass plants as weeds in the crop continue to produce fruits as freely as during the normal period of July to October. With this object in view two experiments were carried out.

In the first ten 4-gallon petrol tins filled with sifted Gezira soil were sown monthly, each with three nut-grass tubers dug up from the soil of the Gezira Research Farm. The tubers were chosen as being of similar size and apparently alive, and were sown on the same day as they were removed from the soil. The first set of tubers was sown at the beginning of April 1939 and the experiment continued to December 1941, a total of 33 months. The tins were watered daily. Not all the tubers germinated and monthly totals of germinated tubers varied from 20 to 30. Records were made of the number and date of appearance of the flowers. This date was arbitrarily chosen to be that on which the flowers exposed their stamens. Observations on each monthly set of tins were continued in most cases until 5 months after sowing of the tubers.

TABLE II  
*Monthly Production of Flowering Heads (Mean per Tuber  $\times 100$ )*

Month of Sowing	1939					1940					1941				
	Month after Sowing					Month after Sowing					Month after Sowing				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Jan.	—	—	—	—	—	0	0	0	0	0	0	70	0	0	0
Feb.	—	—	—	—	—	0	0	0	0	0	0	0	0	0	14
Mar.	—	—	—	—	—	0	0	0	0	29	0	0	0	16	20
April	104	750	285	0	0	0	0	52	376	86	0	3	20	16	90
May	42	358	375	21	0	0	0	192	208	54	0	10	50	115	25
June	408	75	4	0	0	0	473	193	60	0	9	50	127	18	—
July	14	400	111	32	4	100	523	100	0	0	87	360	137	—	—
Aug.	0	220	100	48	4	71	361	0	0	0	50	190	63	0	0
Sept.	0	15	12	4	8	10	185	115	60	25	29	138	76	14	38
Oct.	63	77	0	0	0	90	169	167	17	7	0	20	0	0	0
Nov.	0	3	17	24	0	0	73	10	3	—	0	0	0	0	0
Dec.	0	0	4	0	0	10	20	3	—	—	0	73	35	46	—

Table II shows the monthly appearance of flowering heads expressed as the mean per tuber  $\times 100$ . It will be observed that the greatest number of flowering heads is formed when the sowing month occurs between April and October and that tubers sown from November to March produce few flowering heads. The April to June sowings (inclusive) of 1940 show that the month of maximum flowering occurs nearer and nearer to the sowing month as that month approaches the July–October period, i.e. the rainy season. There is some additional evidence of this in the 1941 series. The April sowing of 1939 for some unknown reason behaved anomalously, but later sowings also tend to show this character. Fig. 1 shows the total production of flowering heads (expressed as the logarithm of the mean production per tuber  $\times 100$ ) during the 1st, 2nd, and 3rd month after sowing, arranged according to the month in

which the 1st, 2nd, and 3rd month after sowing would occur. The bottom curve shows the monthly average of relative humidity (8 a.m.) as given by the instruments in a standard meteorological screen. It will be noticed that the greater proportion of the production occurs during the rainy season, while in no case after April 1939 is a flowering head produced during March and April. The logarithmic curve, while condensing the figures to make them suitable for graphing, tends to hide the size of the differences between the production totals. This is best shown by Table II. It is clear that though the bulk of the production occurs during the rainy season, irrigation permits some

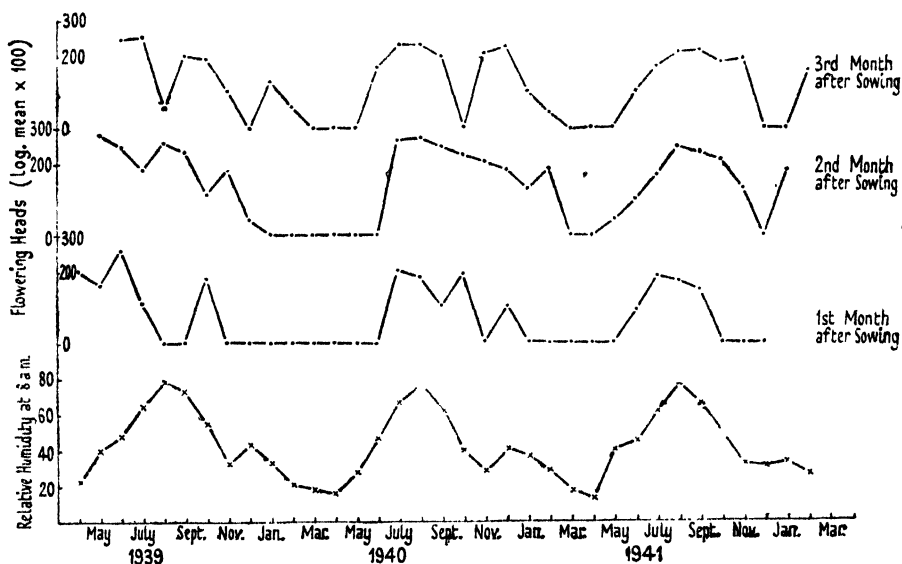


FIG. 1. Total production of flowering heads for first, second, and third month after sowing; results expressed as mean heads per tuber  $\times 100$ . Bottom curve the monthly average of relative humidity.

flowering heads to be formed out of season, though they are small in number compared with the production during the rainy season. A regression coefficient calculated between the monthly production of flowering heads and the monthly averages for relative humidity at 8 a.m. showed that there was a highly significant relationship between these two factors in the 1st, 2nd, and 3rd monthly production respectively. No significant relationship was found between the monthly production of flowering heads and the monthly average saturation deficit or maximum and minimum temperature (Table IV). It appears that the controlling factor in the production of flowers was the humidity of the atmosphere since the type of soil was the same throughout the experiment, and soil moisture as far as possible was maintained at the same level.

In the second experiment two small unequal plots (called Nos. 47 and 49) having a medium infestation of nut grass were chosen on the Gezira Research

Farm. At the end of September 1941 all fruiting heads were cut from the nut-grass plants, leaving the flowering stalks. Subsequently at the end of each month all fruiting heads were removed and counted and the height of the inflorescence stalks measured. The two plots were watered fortnightly. The experiment was continued until June 1943 (a total of 21 months) when it had to be abandoned owing to excessive growth of perennial grasses the removal of which would have disturbed the nut-grass plants. The fruiting heads were removed monthly so that the production of heads could be related to prevailing weather conditions.

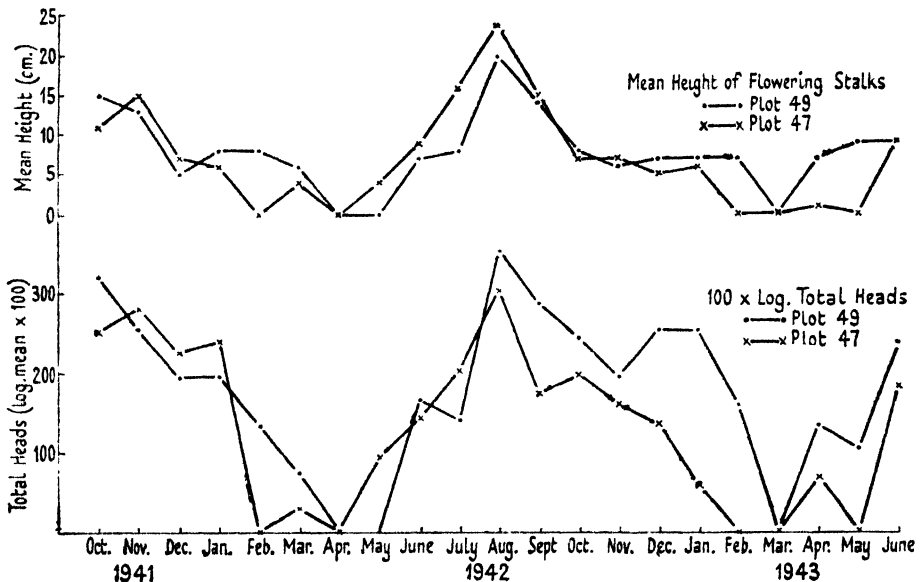


FIG. 2. Curves of mean height of flowering stalk and of total production of flowering heads (log. mean heads per tuber  $\times 100$ ).

TABLE III

*Monthly Production of Fruiting Heads*

Month	Plot 47	Plot 49	Month	Plot 47	Plot 49	Month	Plot 47	Plot 49
1941			1942			1943		
Oct.	332	1259	May	9	0	Jan.	4	334
Nov.	672	367	June	27	49	Feb.	0	41
Dec.	189	90	July	110	25	Mar.	0	0
1942			Aug.	1045	3229	April	5	23
Jan.	174	97	Sept.	58	784	May	0	12
Feb.	0	22	Oct.	98	280	June	67	257
March	2	6	Nov.	43	96			
April	0	0	Dec.	24	339			

Table III shows the monthly production of heads in each plot, while in Fig. 2 is shown this monthly production expressed as the logarithm ( $\times 100$ ) of the total heads and also the mean height (cm.) of the flowering stalks. It

will be observed that the monthly production of heads in the two plots follow a similar curve, and that again maximum production occurs during the rainy season, regardless of the fact that the plants were watered throughout the year. Table IV gives the regression of monthly flowering and fruiting head production of experiments (a) and (b) with different climate factors.

TABLE IV  
*Regression of Flowering and Fruiting Production on Climate*

Monthly averages.	Experiment (a)	Experiment (b) Plot 47	Plot 49
Relative humidity	HS	S	HS
Max. temperature	NS	NS	NS
Min. temperature	NS	NS	NS
Saturation deficit	NS	S(neg.)	S(neg.)

HS = Highly significant.    S = Significant.    NS = Not significant.

Again, it will be noted that no correlation occurs between head production and temperature, but there is a significant regression with relative humidity and saturation deficit. No reason can be given for the fact that no significant regression was found between saturation deficit and production of flowering heads in the first experiment.

Clearly from these two experiments the production of the fruits is governed by the prevailing humidity, and this production reaches a maximum during maximum humidity. It is therefore reasonable to assume that unless the humidity inside a cotton crop is considerably in excess of that prevailing over the surrounding uncropped land, fruit production of nut grass in the cotton will be at a minimum when the atmospheric humidity is low although the cropped land is periodically irrigated.

To determine the relative humidity inside a cotton crop a 5-acre plot was divided in 5 equal blocks. Towards the centre of each block and arranged in a staggered manner through the plot a set of wet and dry bulb thermometers was placed. The cotton, according to normal practice, was sown on 20/8/41 on ridges 80 cm. apart. The sets of wet and dry bulbs were erected on 13/9/41 so that the bulbs were approximately 28 cm. from the ground. Daily readings were taken on each instrument. Fig. 3 shows the average weekly relative humidity (8 a.m.) in the cotton plot, the relative humidity as shown in a standard meteorological screen situated about 1,200 yds. from the plot, and also the average height of the cotton plants in this plot throughout the season. The final height of the cotton plants is somewhat below the normal on the Gezira Research Farm for cotton sown on August 20. It will be observed from this graph that there is little difference between the humidity outside the cotton and that inside until we reach the week ending January 24, after which date the excess humidity in the cotton plot varies from 9 to 18 per cent. It is possible that the measured humidity values in the cotton are somewhat high since the air there is likely to be relatively still, while the instruments in the standard screen would be subject to the slightest breeze. It is necessary

to determine if this excess humidity is sufficient to cause profuse production of nut-grass flowering heads.

Using the data of the second experiment, the regression coefficient for the 1st, 2nd, and 3rd month after sowing when total monthly flowering heads per tuber are correlated with average monthly relative humidity (8 a.m.), is 4.4, 5.8, and 2.6 respectively, yielding an average of 4.3. This value was calculated from the monthly mean flowering heads per tuber  $\times 100$  and repre-

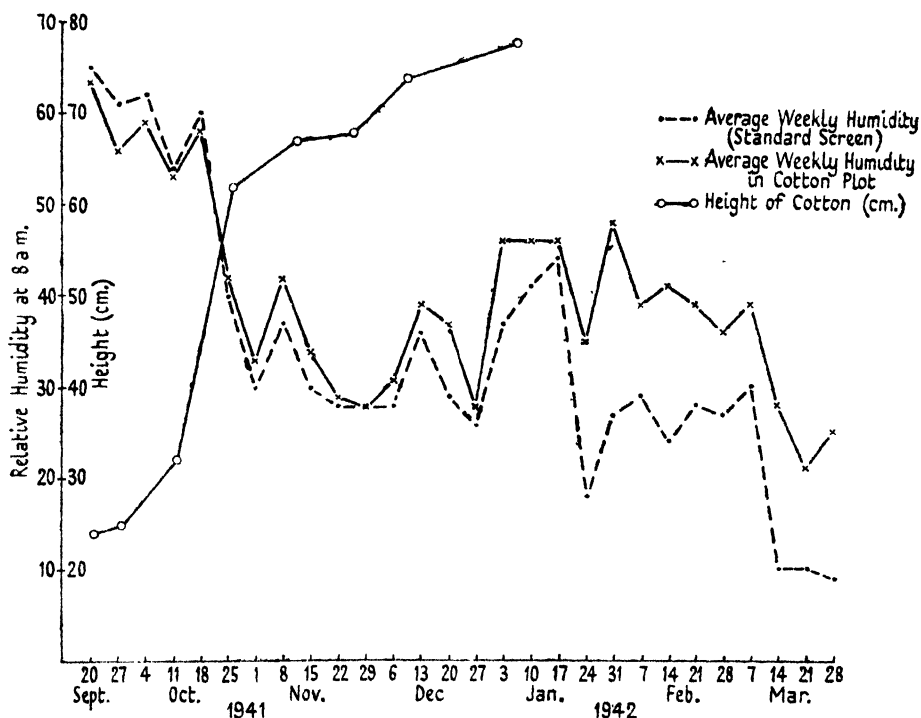


FIG. 3. Average weekly relative humidity (8 a.m.) in cotton crop and in standard screen. The rising curve on the left shows the average height (cm.) of the plants of the plot throughout the season.

sents 100 times the average monthly variation in flowering heads per tuber per unit variation in percentage of relative humidity. The average monthly humidity for the two month period January 24 to March 24 inside and outside the cotton is 36, 21 and 31, 23 respectively, i.e. an excess of 15 per cent. for the first month and 8 per cent. for the second month. From the above data this excess should produce  $15 \times 4.3/100 = 0.6418$  and  $8 \times 4.3/100 = 0.3444$  flowering heads per tuber per month or 64 and 34 on the basis of the figures in Table II. It appears therefore that the increased humidity prevailing in a cotton plot during February and March does cause a small but significant number of flowering heads to be formed during that period. It is conceivable that a very small but continuous production of flowering heads occurs during

the whole period of irrigation, but it is probable that only during February and March, viz. the two months before irrigation ceases, that from the viewpoint of sowing the land with fallen fruits the number of heads produced becomes significant.

In Fig. 2 is shown the mean height (cm.) of the flowering stem throughout the period of observation in plots 47 and 49. It will be noted how closely the two curves follow each other and that the height of the stem increases as the production of heads increases. The greatest production of flowering heads in both plots occurs in August (i.e. the month with the highest relative humidity), and in that month the tallest flowering stems are found. During the rainy season the plant obviously displays its most vigorous growth. Outside that season some inhibiting factor, presumably the lower relative humidity, retards both growth and production of flowering heads.

#### GERMINATION OF THE SEED

When studying the germination of the seed in the field or in the laboratory one is perforce considering the fruit.

Early germination tests on freshly collected fruit using water with and without soil confirmed the results of other workers in that germination was practically nil. It was, however, discovered that if surface soil was collected, sieved to remove all tubers, and the soil watered an abundant crop of nut-grass seedlings appeared. Twenty-five 4-gallon petrol tins (24.5 cm.  $\times$  24.5 cm.  $\times$  34.6 cm.) were filled with sifted surface soil and watered regularly. Nut-grass seedlings appeared. Five tins were washed out at intervals and the number of tubers formed was counted. Table V gives the results obtained.

TABLE V  
*Production of Tubers from Germinated Seed*

No. of days after 1st watering.	Total tubers produced.
30	Nil
47	119
78	179*
110	582
141	1171

\* One tin of this group produced no seedlings.

From Table V it can be calculated that the seed contained in an area of this soil 1 metre square would theoretically produce roughly 400, 800, 2,000, and 4,000 tubers after 47, 78, 110, and 141 days' growth respectively. It is not suggested that this vast production of tubers from seed occurs in the field, but the figures indicate the potentialities under suitable conditions of seed lying in the surface soil. In another experiment of ten petrol tins of sifted soil taken from another area on the Gezira Research Farm the number of seedlings was counted and the depth from which germination had occurred was measured. Table VI gives the results.



TABLE VI  
*Depth Frequency*

Depth (cm.).	No. of seedlings.
0.0-0.5	10
0.6-1.0	23
1.1-1.5	38
1.6-2.0	13
2.1-2.5	16
Below 2.5	0
Total seedlings	100

The non-germination of freshly collected live seed may be caused either by a hard, difficultly-permeable fruit wall since the testa is very thin, or because the fresh seed in the soil is not ripe and has to undergo a period of dormancy before becoming germinable.

In the flooding experiment described earlier (Andrews, 1940, p. 182) it was noticed that 10 days after the flooding ceased in the November-flooded sub-plots germination of the seed was occurring in the spikelets of such ripe flowers as had been held on the ground by the mud. A count of germinated seed on 3-metre squares on each of 3 sub-plots gave totals of 61, 64, and 127 seedlings respectively. Further counts on another 3 metre-squares on the same sub-plots 18 days after the flooding ceased gave totals of 66, 63, and 42 seedlings respectively. It is thus clear that fresh seed is germinable under certain conditions, and shows that for at least some of the seeds a period of dormancy in the soil is not necessary to produce germination.

In a laboratory repetition of this flooding experiment, freshly collected ripe spikes were placed in dishes on river silt and permanently flooded for periods of 7, 16, and 25 days respectively. At the end of each flooding period the water was poured away and the soil kept just moist. Table VII shows the results obtained 49 days after the beginning of the experiment.

TABLE VII  
*Germination of Seed after Flooding*

After 7 days, flooding no germination occurred either during flooding or subsequently.

Length of flooding.	Dish.	Total spikelets.	Total seed calculated.	Germination (%).
16 days	1	364	6006	1.3
	2	354	5190	2.9
	3	394	5735	1.8
	4	318	4201	2.0
25 days	1	448	5913	1.6
	2	301	4080	3.4

Fruits from other spikelets from the same collection sown on blotting-paper at the commencement of the experiment produced no seedlings after

29 days. It would appear that association with wet soil is necessary for germination, and that a difficultly-permeable fruit wall has to be softened under the action of bacteria in the soil. The very low percentage germination obtained in all the experiments is noteworthy.

Attempts were made by scarifying the fruit and also treating it with strong sulphuric acid for 15 min. to increase the germination of the seed. A certain measure of success was obtained with sulphuric acid, but the highest germination averaged only 11 per cent. Scarification in our experiments appeared to injure the seed. Ranade and Burns (1925) record that heating the fruits for one hour to 139° F. (58° C.) increased germination. The temperature of the surface soil of the Gezira rises considerably above this temperature on many days of the year. Ranade and Burns (1925) also state that 'soil moisture without atmospheric humidity will not cause germination, and in the field the rainy season is therefore the only one when much germination of seeds occurs'.

A series of experiments was instituted to discover if the germinating power of the seed increased with increasing age of the fruit. Fruits collected in different years were sown for successive seasons. For each year of collection of the fruit twenty-five 4-in. pots were nearly filled with sifted surface soil which had been heated for 24 hours at 120° C. The object of the latter was to destroy any seed in the soil. A thousand nut-grass fruits were sown on blotting-paper in each pot and covered with about  $\frac{1}{8}$  in. of heated soil. There were thus 25 replicates of each year of collection, giving a total of 25,000 fruits in each sowing. The sown pots were prepared in January of each year and left in the open exposed to the sun until June 20-5, after which date they were watered twice daily. The sown pots were exposed in the open from January to June with the intent to counteract the sterilization of the soil caused by the original heating, and to restore it as far as possible to a condition similar to that of the surface soil of the surrounding land. The germinated seed was counted and removed every 2 days. The fruits collected in September of each year were stored before sowing in loosely corked bottles in the laboratory. Table VIII gives the percentage germination 2 months after the first watering, by which time all further germination had ceased.

TABLE VIII  
*Germination Percentage*

Year of collection.	Year of Sowing.		
	1942.	1943.	1944.
1937	10.5	30.8	43.4
1939	21.5	26.7	51.9
1941	6.7	13.8	29.3

An analysis of variance on the results showed that all differences except that between 1937/43 and 1941/4 were highly significant.

A similar experiment to the above was arranged where the fruits instead of remaining in the pots until June before being watered were watered at the beginning of February. Fruit from the same three years of collection was used, but the experiment was carried out only in 1942 and 1944. Table IX shows the results obtained two months from the date of sowing.

TABLE IX  
*Germination Percentage*

Year of collection.	Year of sowing.	
	1942.	1944.
1937	18.4	25.0
1939	10.4	26.1
1941	1.9	18.1

An analysis of variance showed that all differences were highly significant except those between 1937/42 and 1941/4, and 1937/44 and 1939/44.

It is clear that increasing age of the fruit causes increased germination of the seed. Since fruits were used in the experiment it is impossible to decide if the increased germination was due to the ripening of the seed, or to the fruit wall becoming more permeable. To the naked eye there appeared to be no external difference between the fruit collected in 1937 and that collected in 1944. The fruit was stored in loosely corked bottles in the laboratory and it would be expected that no fundamental change in the permeability of the fruit wall would occur. By inference it would appear that a period of dormancy is necessary to ripen the bulk of the seed, but there is as yet no direct evidence of this, since it is impossible to experiment with the seed without its fruit wall.

The average relative humidity (8 a.m.) for February, March, and April is 26, 20, and 17: in fact they are the driest months of the year. In spite of this 25 per cent. germination was obtained in the second experiment (Table IX). This does not support the findings of Ranade and Burns (1925) that soil moisture without atmospheric humidity will not cause germination of the seed or that in the field the rainy season is therefore the only one when much germination of seed occurs. All the pots were in the open and only differed from the surrounding land in that the pots were watered.

The oldest seed, viz. that collected in 1937, showed after 7 years' storage its maximum germination of 43 per cent. and provides information on the longevity of the seed of this plant.

Goss (1924) has described a series of experiments, commenced by Duvel, on the vitality of buried seeds. Seeds representing 107 species were mixed with sterile soil in flower pots and each species was buried at depths of 8 in., 22 in., and 42 in. The experiment was started in 1902. Only one member of the Cyperaceae, viz. *Cyperus esculentus* L., was included in the series and 200 fruits of it were buried at each level. The following table shows the results obtained by Goss.

TABLE X

Depth of burial (in.).	Germination percentage.					
	1903.	1905.	1908.	1912.	1918.	1923.
8	0	1.5	4.5	7.0	0.5	8.5
22	0	2.5	5.5	21.0	5.0	5.0
42	0	1.5	0.0	14.0	0.0	17.0

It will be observed that up to 1912, i.e. up to 10 years after the experiment started, the germination percentage, except 1908 at the 42-in. level, steadily increased. The results therefore conform with those described above for *Cyperus rotundus* L. It would be interesting to discover if fruits of Cyperaceae in general show, within limits, increasing powers of germination with increasing age. This increased germination with increasing age probably accounts for the lack of germination in freshly collected fruits and supplies an explanation for the heavy germination obtained in watered surface soil, which must contain fruits of different ages.

It was noticed that the fruit of nut grass was covered with a waxy substance. This substance was soluble in ether and alcohol and had a fairly sharp melting-point of  $81^{\circ}$  C. To test if this waxy covering was inhibiting germination, fruits of the 1944 crop were washed with ether and with alcohol. Five hundred fruits of each of the three treatments, ether-washed, alcohol-washed, and control, were sown each in one small pot in the manner described previously. The experiment was conducted in the open, but the pots were contained in a large glass-sided chamber where arrangements were made to maintain a constantly high humidity. There were 13 replicates of each treatment with a total of 6,500 fruits per treatment. The pots were watered on 26/10/44. By 23/11/44 the total number of germinated seeds in each treatment was, ether-washed 137, alcohol-washed 123, and control 178. The differences between the total germinated seed in each treatment were not significant. It is clear that the removal of the waxy substance had not assisted germination by increasing it above that usually found in freshly collected seed.

To determine if the fruit wall had first to be acted on by soil bacteria before ready germination occurred, the following experiment was carried out. Eighty small pots were filled with river silt that had been sifted through a sieve fine enough to retain any nut-grass seeds. Forty pots with their soil were heated to  $120^{\circ}$  C. for 24 hours to sterilize both pot and soil. The treatments were as follows: (a) *Heated soil*. Each pot sown with 500 fruits of the 1941 crop. (b) *Heated soil*. Each pot sown with 500 fruits of the 1944 crop. (c) *Non-heated soil*. Each pot sown with 500 fruits of the 1941 crop. (d) *Non-heated soil*. Each pot sown with 500 fruits of the 1944 crop.

There were 20 replicates, with a total of 10,000 fruits in each treatment. The pots were sown on 12/10/44 and contained in the moist chamber described in the previous experiment. Watering was with boiled water. It was realized that only partial sterilization of the soil would be obtained by

this means, but it was expected that the sterilization would be sufficient to produce a time-lag in the germination of the seed in the heated pots. Table XI shows the total percentage germination of the 1941 and 1944 fruits 41 days after sowing. Fig. 4 shows the progressive increase of the germination. It will be noted that there is a considerably greater germination in the 1941 seed

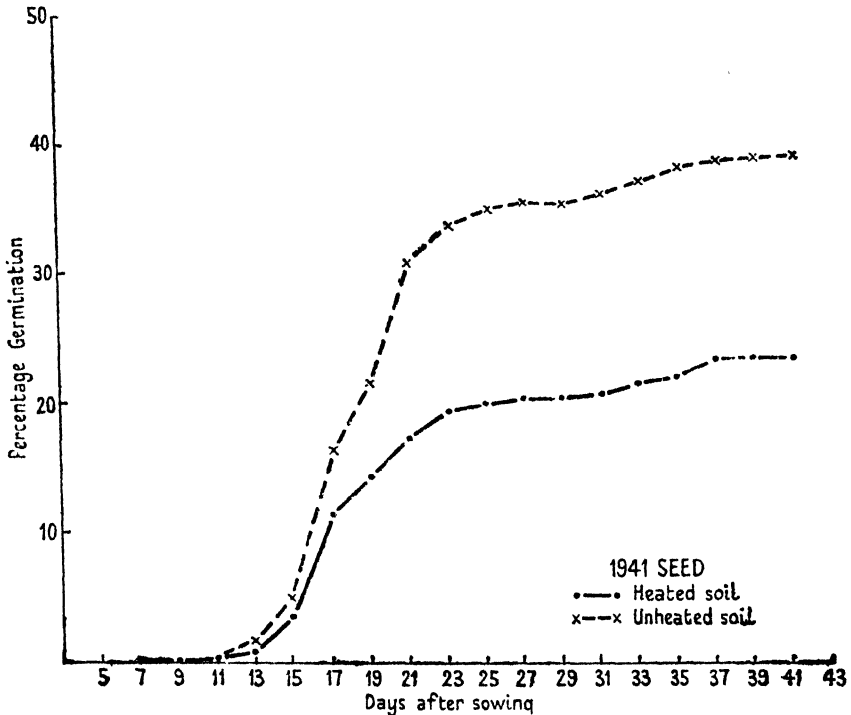


FIG. 4. Graphs showing germination of 1941 seed in heated and unheated soil.

TABLE XI

Heated soil.		Non-heated soil.	
1941 Fruit.	1944 Fruit.	1941 Fruit.	1944 Fruit.
23.9	1.2	39.3	1.3

in non-heated soil than in the heated soil. The difference 35 days after sowing is highly significant and is evidence that rotting of the fruit wall is necessary for germination. The soil in the pots was kept very moist, and the effect of the partial sterilization was shown in that the soil of the non-heated pots was covered with a scum of green algae, while in those pots containing heated soil the surface soil was almost without this green covering. The difference between the germination of the 1944 fruit in non-heated and heated soil was not statistically significant.

Summarizing the experiments on the germination of nut grass seed it appears that the bulk of fresh seed has to undergo a period of ripening after it is shed before it becomes germinable; but when that period is complete germination is hastened by any treatment that includes disintegration of the fruit wall. It is noteworthy that germination can be obtained even in fresh seed, though only in very small quantity. Why this small quantity of fresh seed is germinable and not the bulk of the seed is not known. It suggests that it is possible in a limited degree for the ripening process to occur while the seed is still on the plant.

#### DISPERSAL OF NUT GRASS FRUITS

On land not bearing a crop there should not be much widespread dispersal of nut grass fruits. During the rains, when nut grass plants are in full flower, the land is covered with weeds which tend to over-top the nut grass plants and the fruits have little opportunity to spread even with the help of winds. Only when the plants lie in the path of the small cyclonic winds, locally known as 'dust devils', is wide dispersal likely to happen. Widespread dispersal is also likely to occur from low-lying land, or from the banks of irrigation canals and watering channels where nut grass is often the dominant weed. In the cotton plots the cotton will act as an effective screen. A potent source of dispersal of the fruits is the irrigation water. As has previously been stated the plant lines the banks of the canals often to the water's edge. Fruits are shed on to the irrigation water, and owing to the fact that they lie on the surface of the water for some time before sinking there is ample opportunity for them to be carried by the water through the field outlet pipes on to the cultivated land. During the period September 1943 to March 1944 water was collected as it issued from the field outlet pipes, i.e. immediately before it flowed on to the land. The water was filtered through a mesh small enough to retain nut-grass fruits. A total of 3,479.5 cub. m. (773,221 galls.) of water was examined, taken from 18 outlet pipes, the bulk of which were near the Gezira Research Farm. This quantity of water yielded 1,136 nut-grass fruits. One outlet pipe waters on an average 90 acres of cropped land and passes roughly 36,000 cub. m. of water at each watering. There are about 14 waterings per season, so that a total of roughly 504,000 cub. m. are required to bring 90 acres of cotton to maturity. From the above figures this quantity of water would contain 164,520 nut-grass fruits or very roughly 2,000 fruits per acre. The quantity of fruits found per month varied greatly as did also the volume examined, and no month appeared to provide much more fruits than another. This is somewhat surprising since the maximum outburst of fruiting occurs between August and November. The quantity of nut-grass plants in the lengths of canal which fed the different outlet pipes differed considerably and the above figure of nut-grass fruits can only be very approximate. The figure, however, gives some idea of the rate of dispersal of the fruits by irrigation water.

## RE-INFESTATION OF THE GEZIRA LAND BY NUT-GRASS SEED

Observations made in the Gezira on seed germination in the field showed that very few seedlings reach maturity. A series of observations was made in the southern part of the Gezira, where the rainfall is somewhat heavier than that at the Gezira Research Farm. Land not bearing a crop and therefore receiving water only from the rains was examined for nut-grass seedlings during the rainy season. The observations showed that in low-lying places where water was liable to collect abundant seed germination occurred, but that on the bulk of the land while germinated seed was found the seedlings soon dried out and withered. Germination took place in the top 2-3 cm. of soil, and it appeared that successive showers were too widespread in time to prevent the periodic drying out of this thin layer of soil. On irrigated land the position is somewhat different. The cotton crop is in general sown between the 15th and 25th of August. The land has therefore been subject to the July rains and those of the first half of August before the sowing commences. Immediately prior to the sowing the land is hoed to remove all weeds. Thus the top surface of the soil is disturbed and the majority of the nut-grass seedlings produced by the rains would in consequence die. Further rains tend to produce another crop of nut-grass seedlings, but further hoeing of the land to remove the new weed growth from the young cotton plants would also kill most of the nut-grass seedlings. After this second hoeing, the cotton ridges are remade by means of a ridging plough travelling along the old furrows. Extensive killing of the nut-grass seedling occurs at this operation. In one small area under observation 84 seedlings were reduced to 2 by this re-ridging. These cultural operations are usually finished by mid-October. It is only after that date that the seedlings remain relatively undisturbed to survive under irrigation, while further quantity of germination appears to lessen as the drier weather approaches. Examination after mid-October of fourteen 5-acre cotton plots on the Gezira Research Farm revealed a total of only 214 nut-grass seedlings, of which 19 survived to the beginning of the following March, and produced a total of 8 tubers. These tubers would presumably survive to the following rains when multiplication would be rapid. It is thus apparent that owing to the prevailing climate conditions annual re-infestation of the Gezira cotton area by nut-grass seedlings does not occur to any great extent. Seedlings do survive and form tubers, but the quantity is so small that they are of little importance when dealing with the far more important problem of eradicating the tubers of this plant.

## SUMMARY

The distribution of nut grass in the Gezira and a description of the flowering head of this plant is given.

It is shown that the production of flowering heads of nut grass in the field is governed by the prevailing relative humidity, maximum production occurring at periods of maximum humidity.

### 30 *Andrews—Study of Nut Grass in Cotton Soil of the Gezira. II*

The increased humidity inside a cotton crop is only able to produce a small increased production of flowering heads.

The length of the flowering stem varies with relative humidity, maximum length with maximum humidity.

It is shown that increased germination is obtained with increasing age of the seed.

The lack of germination in freshly collected seed is most probably due to the need for the seed to undergo a period of dormancy.

Some details of fruit dispersal of nut grass in the Gezira are given.

It is shown that annual re-infestation by seed does not occur to any important extent in the Gezira.

I wish to acknowledge the assistance I have received from Zein Eff. Abdel Nabi, our Senior Technical Assistant, and other members of the staff in the collection of the data.

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# Studies in the Physiology of Leaf Growth

## II. Growth and Structure of the First Leaf of Rye when cultivated in Isolation or attached to the Intact Plant

BY

R. S. DE ROPP

*(Research Officer, Agricultural Research Council, Rothamsted Experimental Station, Harpenden)*

With six Figures in the Text

### INTRODUCTION

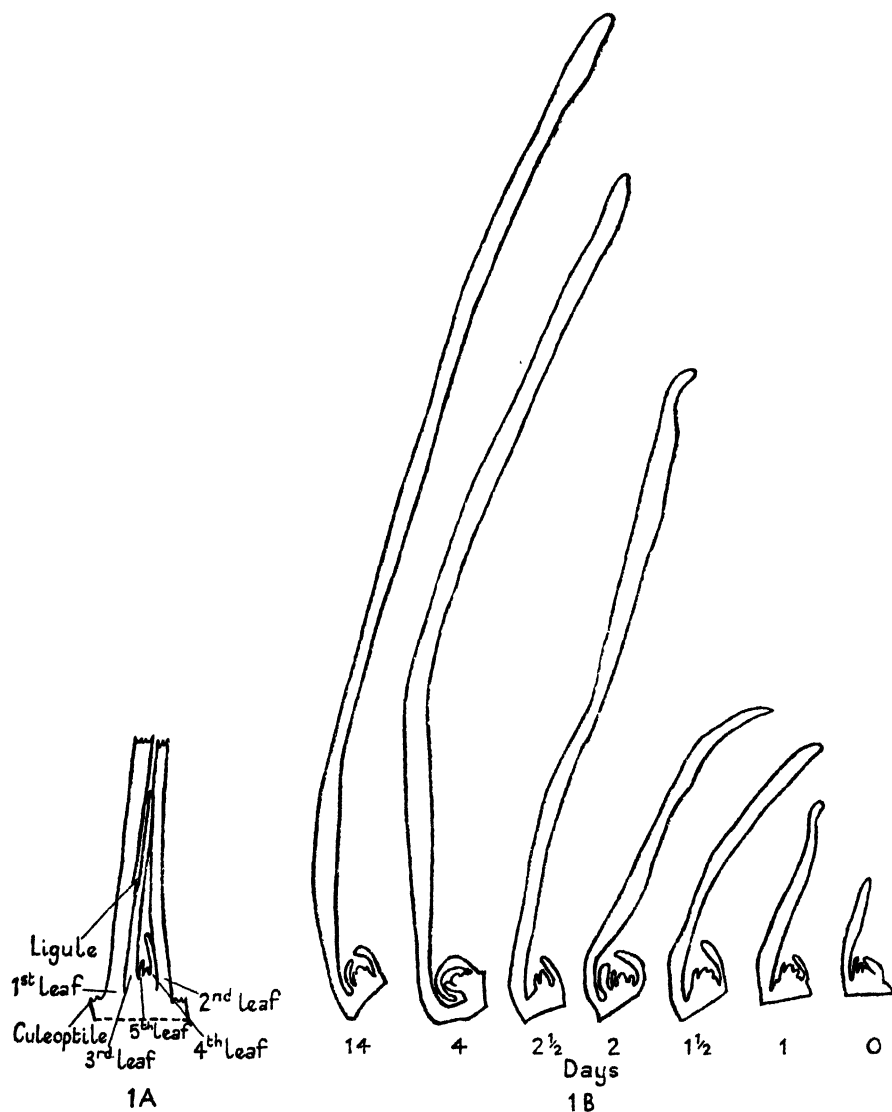
**F**EW workers have studied the processes of leaf growth and differentiation in the Gramineae. Apart from some early observations of Douliot the main contributions on this subject were made by Rösler (1928) and Kliem (1936), who described the mode of origin of the leaf at the growing-point in *Avena* and *Triticum*. The external morphology of the shoot apex in various grasses and cereals was studied by Sharman (1942), who distinguished three main types of apex. The growth and development of the shoot in *Sinocalamus* has been described by Hsü (1944). Percival (1921) has given a fairly detailed description of the structure of the mature leaf in *Triticum*.

The purpose of the present study was to follow the process of differentiation in the first leaf of rye both when attached to an intact plant and when cultured in isolation on sucrose-mineral agar, as previously described (de Ropp, 1945). In particular an answer was sought to the following questions: (i) Is the limited growth of the first leaf attached to an isolated stem tip due predominantly to an increase in cell number or in cell size? (ii) How does the structure of the first leaf grown in isolation compare with its structure when grown attached to the plant? (iii) How is growth and differentiation distributed in isolated and attached leaves?

### MATERIALS AND METHODS

Stem tips excised from rye embryos were cultured at a constant temperature of 25° C. in 50-ml. flasks containing sucrose-mineral agar. Intact rye grains were grown in 100-ml. flasks on the same medium without the sucrose. Material for histological study was fixed for 24 hours in Bouin's solution and embedded in paraffin wax (m.p. 52° C.). Sections were usually cut to 8  $\mu$  and stained by means of Newton's gentian violet. Drawings were prepared by projecting the microscope image on to a sheet of paper and drawing in the cells.

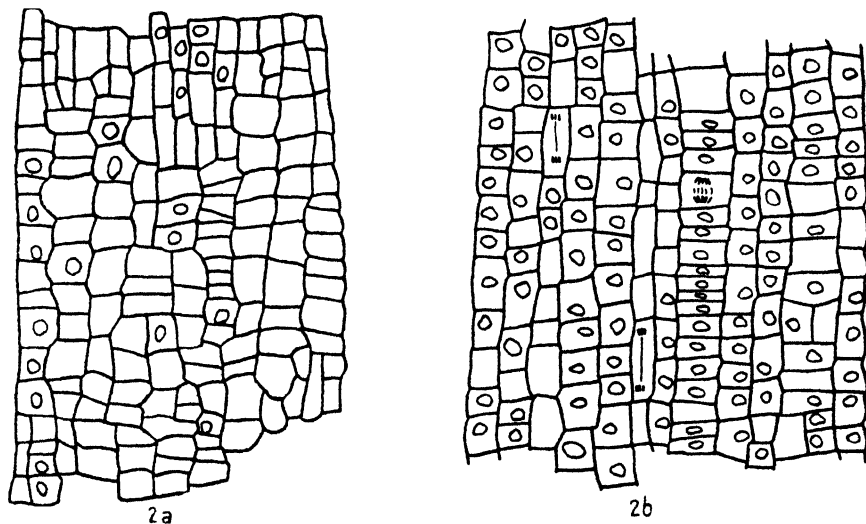
The distribution of growth in the first leaf was studied by carefully stripping the coleoptile from the embryo prior to germination and marking off the



FIGS. 1A and 1B. 1A. Growth during first 14 days of isolated stem tips cultured on nutrient agar, showing limited expansion of the first leaf and lack of development of younger leaves. (Median longitudinal sections.) ( $\times 30$ .) 1B. Median longitudinal section of 3-day-old intact embryo, showing development of younger leaves. First and second leaves truncated and coleoptile removed. ( $\times 14$ .)

exposed leaf primordium into four equal segments. Marking was carried out under a binocular microscope, a single hair dipped in a mixture of vaseline and lamp-black being used for the purpose. These marks had to be renewed

at intervals as growth proceeded. Each segment marked off measured from 0.2 to 0.3 mm., the total length of the primordium being about 1 mm. The distance of these points from the point of attachment of the leaf was determined at daily intervals after germination by means of a travelling microscope. It was found that the above treatment, when performed carefully, did not interfere with leaf growth. Any specimens showing abnormal growth were rejected.



FIGS. 2a and 2b. Longitudinal sections through the base of the  $1\frac{1}{2}$ -day leaf. 2a. Leaf attached to isolated stem tip. 2b. Leaf attached to intact plant. ( $\times 220$ .)

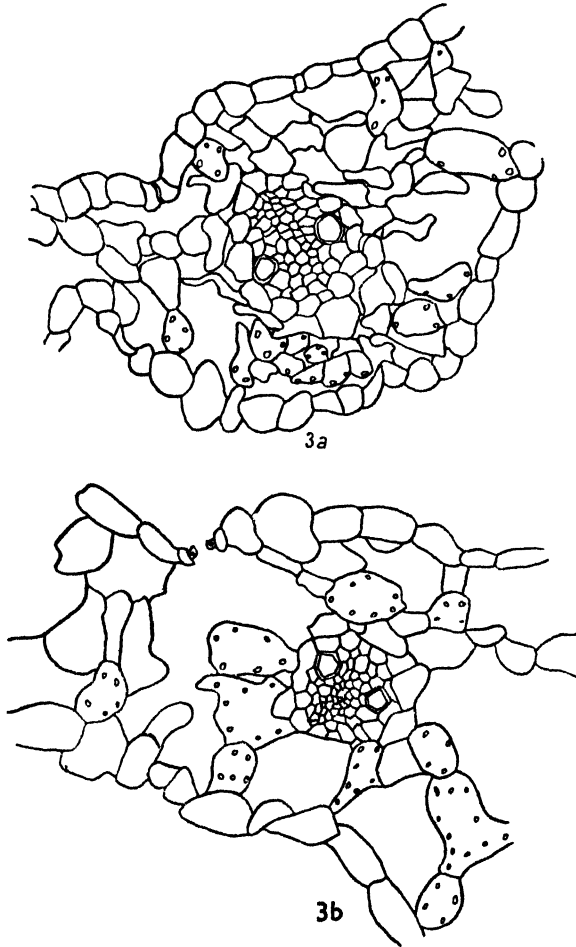
## RESULTS

### (i) *Structure of the isolated first leaf compared with that of the first leaf attached to the intact plant.*

It was shown previously that a stem tip excised from a rye embryo and cultured on sucrose-mineral agar is capable of making a limited amount of growth which is almost entirely confined to the first leaf. This finding was confirmed in the course of the present work. In Fig. 1A are shown a number of median sections of isolated shoot tips cultured for different times on sucrose-mineral agar. A comparison of the appearance of these sections with that of a 3-day-old intact embryo (Fig. 1B) shows the extent to which the growth of younger leaves has been inhibited in the detached stems.

Sections of isolated stem tips cultured on nutrient agar were examined to discover whether the limited growth of the first leaf primordium was entirely due to an expansion of already existing cells or whether any increase in cell number had also taken place. Fig. 2a shows a drawing of the basal region of the first leaf on an isolated stem tip after  $1\frac{1}{2}$  days' growth. No mitotic figures were visible in the section and the cells were no longer of the meristematic type. The same region of the first leaf on an intact plant is shown in Fig. 2b.

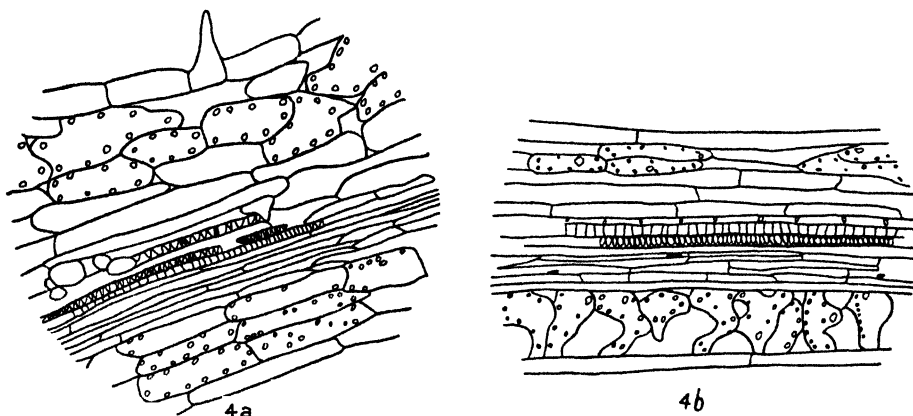
Here the signs of active cell division were obvious. The whole basal region of the leaf was meristematic. Many mitotic figures were visible; the cells were small, laid down in regular series, with dense unvacuolated cytoplasm and deeply staining nuclei. Since a careful examination of many sections of isolated first leaves failed to reveal any signs of recent cell division, it was concluded



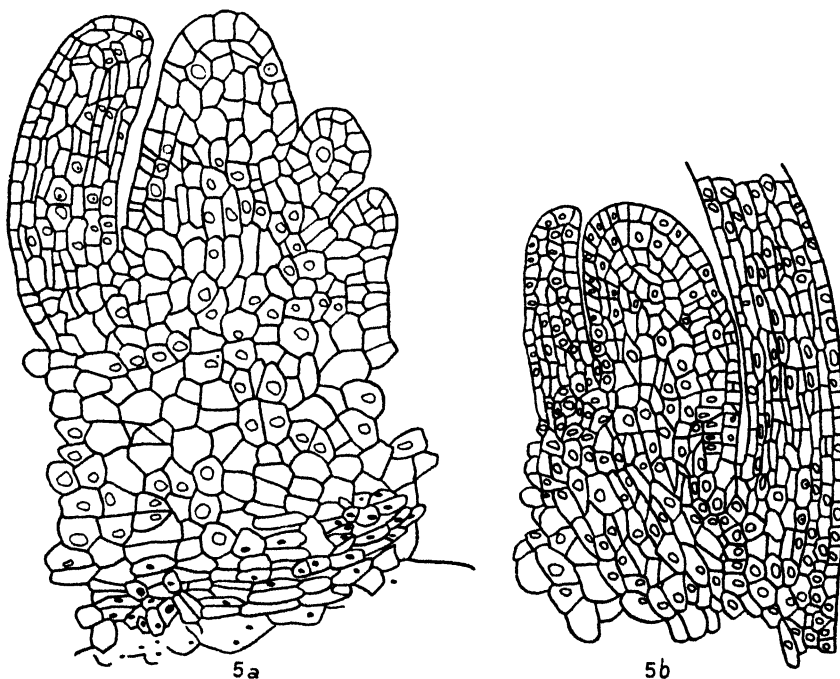
FIGS. 3a and 3b. Transverse sections through the lamina of a 14-day leaf of rye. 3a. Leaf attached to isolated stem tip. 3b. Leaf attached to intact plant. ( $\times 200$ .)

that the limited amount of growth made by these leaves was due entirely to an increase in size of the already existing cells. Cell measurements showed that the length ratio of an embryonic epidermal cell to a mature epidermal cell was about 1 : 15. The length ratio of the embryonic leaf primordium to the fully grown leaf cultured in isolation was about 1 : 18. Cell elongation would thus appear to be sufficient to account for the increase in length of the leaf. This absence of cell division in the isolated stem tip of rye contrasts with the

behaviour of the isolated stem tips of *Stellaria media* described by White (1933), who succeeded in tracing the actual course of cell division in some of the stem tips he studied.



FIGS. 4a and 4b. Longitudinal sections through the lamina of a 14-day leaf of rye. 4a. Leaf attached to isolated stem tip. 4b. Leaf attached to intact plant. ( $\times 150$ .)

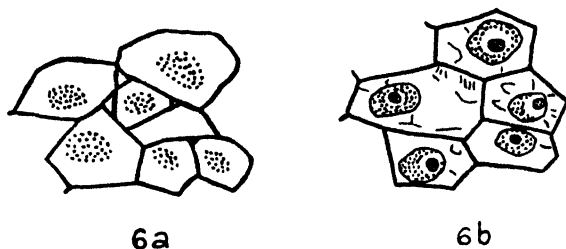


FIGS. 5a and 5b. Median longitudinal sections through the stem tip of 14-day-old rye plants. 5a. Isolated stem tip. 5b. Intact plant. ( $\times 200$ .)

Transverse and longitudinal sections of the isolated and attached leaf (Figs. 3a, b, and 4a, b) did not reveal any obvious differences in structure in the mature leaf tissues. The appearance of vascular bundles in cross-section

(Fig. 3*a* and *b*) was that of a typical monocotyledonous leaf. The cells of the leaf parenchyma and of the epidermis are slightly smaller in the isolated leaf than they are in the attached leaf. As may be seen from Fig. 4*a* and *b*, the vessels in these leaves are for the most part spirally thickened.

A comparison of the internal structure of the growing-point in a 2-week-old isolated stem tip and a stem tip attached to the plant reveals several differences in cell structure which are shown in Figs. 5 and 6. In the isolated growing-point (Fig. 5*a*) the cells have ill-defined nuclei which stain weakly and show no signs of a nucleolus (Fig. 6*a*). The cell walls are uneven and also stain weakly. At the base of the isolated stem tip a zone of dead and partly dis-integrated cells can be seen, still embryonic in size with deeply staining nuclei (Fig. 5*a*). By contrast, the cells of the attached stem tip are well developed with



FIGS. 6*a*. and 6*b*. Cells from the stem tip of 14-day plants. 6*a*. Isolated stem tip. 6*b*. Intact plant. ( $\times 600$ .)

clearly defined nuclei, deeply staining nucleoli and firm cell walls (Fig. 6*b*). The cells in the actively growing regions (Fig. 5*b*) are considerably smaller than those of the detached stem tip.

(ii) *Distribution of length increase in the first leaf when attached to an isolated stem tip or intact plant.*

It was not found practicable to divide off the isolated first leaf primordium into segments owing to the difficulty of performing this operation with sterile materials. The overall length increase of a group of isolated leaves was therefore measured at daily intervals, and the mean values of these measurements are shown in Table I.

TABLE I

*Daily Increase in Length of the isolated first Leaf cultured on Sucrose-Mineral Agar in Darkness*

	Age (days).								
	0	1	2	3	4	5	6	7	8
Length (mm.)	1.1	2.0	5.0	9.8	14.0	16.0	17.2	18.5	18.5

These figures agree with those given in an earlier paper showing that growth in length of the first leaf is completed under these conditions within 7 days from the beginning of growth.

Leaf primordia which had been marked off into four approximately equal segments by the method previously described were grown both in darkness and in intermittent light (12 hours of light alternating with 12 hours of darkness). The length of each segment of the leaf was measured daily, and the values obtained are given in Table II.

TABLE II

*Growth in Length (mm.) of the First Leaf attached to the Intact Grain cultured in Light and Darkness*

Segment.	IN LIGHT								
	Age (days).								
	0	1	2	3	4	5	6	7	8
Tip (4)	0.3	0.9	3	3	3	3	3	3	3
(3)	0.3	0.8	5	7	7	7	7	7	7
(2)	0.3	0.9	3	13	14	15	15	15	15
Base (1)	0.3	0.5	3	24	50	63	71	77	77
Total	1.2	3.1	14	47	74	88	96	102	102

IN DARKNESS									
	0	1	2	3	4	5	6	7	8
Tip (4)	0.3	1.0	3	4	4	4	4	4	4
(3)	0.2	0.4	1	4	9	9	9	9	9
(2)	0.3	0.5	1	5	17	84	91	95	95
Base (1)	0.3	0.3	1	11	32	72	117	131	132
Total	1.1	2.2	6	24	62	169	221	239	240

These figures show clearly the extent to which elongation in rye is concentrated towards the basal portion of the leaf. The upper segment appeared from sections to receive no further increment of cells during its period of growth. Its increase in length was apparently entirely due to elongation and differentiation of cells already present in the embryonic primordium. The nearer a segment was situated towards the base of the leaf the larger was the increase in the number of its cells. Sections showed that the meristematic region of the leaf was situated slightly above its point of attachment. Differentiation of the tissue began at the tip of the leaf and progressed towards the base. The absence of mitotic figures from sections of leaves which were more than 3 days old suggested that the total number of cells in the leaf had already been completed by the third day after germination. Subsequent growth resulted not from the production of new cells but from the progressive elongation and differentiation of cells already laid down. It appears, therefore, that the ultimate size of the leaf is largely regulated by the activity of the basal meristem during the first 3 days of growth.

The figures given in Table II also provide information about the effect of light on the elongation of the leaf. Elongation was most rapid in leaves grown in the light up till the third day after germination. Subsequently, the leaves

grown in darkness elongated more rapidly and attained a final length of more than twice that of the leaf cultured in the light. This finding has a bearing on the view, already suggested, that leaf growth during the first 3 days after germination is due mainly to an increase in cell number, whereas subsequently it is due almost entirely to an increase in cell length. It would be during the phase of cell elongation that an exposure to light might be expected to reduce growth in length owing to its effect on the hormone mechanism controlling cell elongation.

No sign of a leaf sheath could be seen in leaves attached to an isolated stem tip. In the leaves attached to the whole plant the formation of the sheath was indicated by the differentiation of the ligule during the second day after germination. The ligule resulted from a group of periclinal divisions in certain cells of the inner epidermis. As soon as it could be clearly distinguished from the lamina the leaf sheath was also marked off into four approximately equal segments and the length of these segments measured at daily intervals. These values are shown in Table III.

TABLE III

*Growth in Length (mm.) of the Leaf Sheath of the First Leaf of Rye attached to the Intact Plant*

Segment.	IN LIGHT					IN DARKNESS				
	Age (days)					Age (days)				
	3	4	5	6	7	3	4	5	6	7
Tip (1)	0.7	2	4	5	5	1	4	6	11	11
(2)	0.8	1	4	7	7	1	3	6	10	13
(3)	0.7	1	3	6	9	1	2	8	14	17
Base (4)	0.8	1	3	6	7	1	2	6	9	10
Total	3.0	5	14	24	28	4	11	26	44	51

It will be seen that the distribution of growth in the leaf sheath differs considerably from its distribution in the leaf as a whole. An examination of sections suggested that practically all the cells that go to form the leaf sheath are laid down by the third day after germination, and that further elongation of this organ results in the main from the elongation of cells already present.

For purposes of comparison the distribution of growth in the coleoptile was also studied by marking this organ while still in the embryo into four equal segments and measuring their length after growth had been completed. As can be seen from Table IV the lower half of the coleoptile contributed most to the final length of the organ, but the distribution of growth was unlike that observed in the leaf. No localized basal meristem could be observed in the coleoptile, though some cell divisions did occur during the early stages of coleoptile growth. These findings agree fairly closely with those recorded for the *Avena* coleoptile by Avery and Burkholder (1936).



TABLE IV  
*Growth in Length (mm.) of the Coleoptile of Rye in Darkness*

Segment.	Age of coleoptile.	
	0 days.	7 days.
Tip (4)	0.4 mm.	6 mm.
(3)	0.4 mm.	21 mm.
(2)	0.4 mm.	27 mm.
Base (1)	0.4 mm.	12 mm.
Total	1.6 mm.	66 mm.

### DISCUSSION

It appears from the findings described in this paper that the very limited growth made by the first leaf in rye when attached to an isolated stem tip is due to the failure of the basal meristem to produce new cells. The amount of possible growth of the primordium is therefore limited by the number of cells already present within it. The activity of the basal meristem is responsible for producing by far the greater number of the cells composing the normal mature leaf; restriction of its function must therefore result in a considerable reduction in leaf size. It has always been observed in these experiments that when isolated stem tips developed roots, young leaves attached to the stem tip began to develop. It seems that the roots in some way activate the basal meristem of the leaf, and that in their absence no new cells can be laid down. The nature of this root effect has still to be investigated.

Regarding the mode of growth of the first leaf attached to the plant, these investigations indicate that nearly all the cells are laid down within the first 3 days after germination. The meristem then ceases to be active and further growth of the leaf results from the elongation and differentiation of cells already formed. Differentiation proceeds from the tip towards the base, the ligule and sheath being differentiated last. In the coleoptile no clearly defined meristem could be observed, and the growth of this organ seemed to be mainly due to the elongation of cells already present, though some cell divisions were also observed. The similarity of the distribution of growth in the coleoptile and leaf sheath is of interest and suggests that the coleoptile might be looked upon as a modified leaf sheath.

### SUMMARY

The growth of the first leaf attached to the isolated stem tip of rye was compared with that of the first leaf attached to the intact plant.

Growth of the isolated first leaf was shown to be due to the development of cells already present. In the absence of roots, cell division in the basal meristem of the leaf did not occur.

Growth of the attached first leaf depended mainly on the activity of the basal meristem during the first 3 days after germination.

Differentiation in the attached leaf began at the tip and proceeded towards the base.

No clearly defined meristem was observed in the coleoptile and the growth of this organ seemed mainly due to the elongation of existing cells.

I am indebted to Mr. S. French for help in the preparation of the diagrams for this paper.

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# The Physiology of Plant Growth with Special Reference to the Concept of Net Assimilation Rate

BY

R. F. WILLIAMS

(From the Waite Agricultural Research Institute, University of Adelaide)

With nine Figures in the Text

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## ✓ INTRODUCTION

THE concept of net assimilation rate, or unit leaf rate as it also has been termed, was developed as an aid in the quantitative analysis of plant growth and may be defined as the rate of increase in the dry weight of a plant per unit of active 'growing material'. By the latter is meant any attribute of the plant which is primarily concerned in carbon assimilation and thus has some claim to be taken as a measure of the 'internal factor' for growth. The concept clearly has relevance only during those phases of growth in which carbon assimilation accounts for the bulk of the change in dry weight of the plant.

It has been usual to express rates of carbon assimilation on a leaf-area basis, it thus being assumed that leaf area was an adequate measure of active 'growing material'. Gregory (1917) was the first to calculate net assimilation rates, but it remained for Briggs, Kidd, and West (1920) to formulate appropriate

methods of growth analysis. As their primary measure of the rate of change in weight the latter workers used the relative growth rate and resolved it into two components, the net assimilation rate and the leaf-area ratio. Later contributors in this field have followed this form of analysis, but other bases have been used for the resolution of relative growth rate. Thus Crowther (1934), Ballard and Petrie (1936), Williams (1936), Heath (1937 and 1937*a*), and others have substituted leaf weight, usually because the accurate measurement of leaf area proved impracticable. More recently Williams (1939), Tiver (1942), Tiver and Williams (1943), and Petrie and Arthur (1943) have used leaf protein-nitrogen as an alternative measure of the active 'growing material'. Since the three leaf attributes named are rarely proportional to each other it is important to know to what extent they fulfil the requirements of the definition of net assimilation rate, and to be aware of their limitations for any specific case. The primary purpose of this paper, therefore, is to discover which, if any, of these attributes is a satisfactory index of the 'internal factor' for growth. To this end a critical review of the more important contributions in the field of plant-growth analysis is made, the results of a further growth experiment are presented, and an attempt is made to assess the value of the concept of net assimilation rate for the furtherance of our knowledge of the physiology of plant growth. Before proceeding with the review, it is proposed to re-examine the derivation of the formulae for relative growth rate and net assimilation rate.

### SYMBOLS

$R$  = Relative growth rate.

$E$  = Net assimilation rate.

$E_A$ ,  $E_W$ ,  $E_P$ , and  $E_N$  = Net assimilation rate on basis of leaf area, of leaf weight, of leaf protein-nitrogen, and of leaf total-nitrogen.

$W$  = Dry weight of the whole plant.

$L$  = Any leaf attribute used as a measure of the active 'growing material'.

$L_A$ ,  $L_W$ ,  $L_P$ , and  $L_N$  = Leaf area, leaf weight, leaf protein-nitrogen, and leaf total-nitrogen.

### THEORETICAL CONSIDERATIONS

#### 1. *Relative Growth Rate*

The integrated equation for relative growth rate has been discussed by Fisher (1920), but the corresponding formula for net assimilation rate has not received adequate treatment. Fisher's method for the derivation of mean values of  $R$  over any given time-interval will be restated because of its bearing upon the derivation of  $E$ .

The value of  $R$  at any instant is

$$\frac{1}{W} \frac{dW}{dt} = \frac{d \log W}{dt}. \quad (I)$$

From this it follows that the average value of  $R$  over any given interval, say  $t_2 - t_1$ , is

$$\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \frac{d \log W}{dt} dt = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}, \quad (\text{II})$$

and is independent of changes in relative growth rate during the interval, no assumption being necessary as to the change of  $W$  with time.

If it be assumed that  $W$  increases exponentially with time, then

$$W_2 = W_1 e^{R(t_2 - t_1)},$$

and

$$\log_e W_2 = \log_e W_1 + R(t_2 - t_1).$$

From this

$$R = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

as before. This derivation is also a statement of the 'efficiency index' of Blackman (1919), though Blackman (1920) was aware that  $R$  gives only the *average* 'efficiency index' for a stated period.

Watson and Baptiste (1938, see p. 470) derive equation II by dividing the increase in dry weight per unit time over the interval  $t_2 - t_1$ , by the mean dry weight per plant for this interval.

$$\begin{aligned} R &= \frac{W_2 - W_1}{t_2 - t_1} \div \frac{W_2 - W_1}{\log_e W_2 - \log_e W_1} \\ &= \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}. \end{aligned}$$

The expression,  $(W_2 - W_1)/(\log_e W_2 - \log_e W_1)$  for the mean dry weight per plant can only have been derived by the method set out by Ashby (1937, p. 26) for calculating mean leaf areas, and assumes that  $W$  increases exponentially.

## 2. Net Assimilation Rate

The net assimilation rate at any instant is given by the equation

$$E = \frac{1}{L} \frac{dW}{dt}, \quad (\text{III})$$

and its average value over any given interval,  $t_2 - t_1$ , is

$$\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \frac{1}{L} \frac{dW}{dt} dt.$$

This function, however, is not integrable unless the relationship of  $W$  and  $L$  is known. If it be assumed that the relationship is a linear one (and this does not necessarily imply that both  $W$  and  $L$  are increasing exponentially),  $dW/dL$  will be constant, and the mean value of  $E$  becomes

$$\frac{1}{t_2 - t_1} \frac{dW}{dL} \int_{t_1}^{t_2} \frac{1}{L} dL = \frac{W_2 - W_1}{L_2 - L_1} \times \frac{\log_e L_2 - \log_e L_1}{t_2 - t_1}. \quad (\text{IV})$$

Alternatively, it might be possible to determine the relationship between  $L$  and  $W$  by the method of least squares, and, by substituting for  $L$  in equation III, obtain by integration the true mean value of  $E$  for each successive time-interval. This method demands more extensive data than are usually available; in many cases, too, it would oversimplify the true  $L-W$  relation.

Many workers, including Ballard and Petrie (1936) and Williams (1936), have used equation IV without being sufficiently aware that it is an approximation; in particular, Gregory (1926) and Watson and Baptiste (1938) present the equation, or its verbal equivalent, in such a manner as to suggest that their mean values for  $E_A$  are derived as accurately as are those for  $R$ . They divide the increase in dry weight per unit time over the interval  $t_2-t_1$  by the mean leaf area per plant for this interval (see their parallel method for  $R$  set out above). While the errors introduced by the implicit assumption of linearity in the  $L_A-W$  relation for the 14-day intervals used by Watson and Baptiste may not be serious, their method of derivation is nevertheless misleading, for such errors may become very considerable with longer time-intervals. Williams (1939) and Petrie and Arthur (1943) stressed the fact that equation IV is an approximation, and Williams made corrections for two  $E_P$  values which were seriously in error. The graphical method set out below could have been applied with advantage to all the  $E_P$  values of his paper.

In the experiment to be described, observations were confined to the period of rapid vegetative growth, and from the primary data of Table V it can be shown that the  $L_W-W$  relation is practically linear for all four treatments. Equation IV was thus sufficiently accurate for the calculation of all  $E_W$  values of the experiment. The  $L_P-W$  relation, however, is far from linear (see Fig. 1), and its form differs markedly from treatment to treatment.

In Fig. 1 absolute leaf-protein was plotted against total dry weight, the whole being drawn on a large scale. Smooth curves were drawn through the six experimental values of each treatment, and between each of these six further values were obtained by interpolation. These interpolations were made in accordance with 2-day values of  $W$  obtained from smoothed curves of  $W$  against time.<sup>1</sup> Corresponding values of  $L_P$  were then read from Fig. 1, and  $E_P$  values were calculated for each 2-day interval using equation IV. The  $E_P$  values presented in Fig. 6 are means of the 2-day values for the appropriate harvest-intervals. The efficacy of this procedure for the purpose of eliminating the error involved in the direct use of equation IV is best demonstrated by carrying the procedure a stage farther in the somewhat extreme case of treatment  $P_2N_1$ , harvest-interval 1-5. In this instance interpolations were derived for each day of the 56-day period, and  $E_P$  values were determined for 1-, 2-, 4-, 7-, 14-, 28- and 56-day intervals; the corresponding mean values for the whole period are given in Table 1. The identity of the mean values for 1- and 2-day intervals is fortuitous, but the data as a whole indicate that  $E_P$  tends to a constant mean value as  $\Delta t$  tends to zero. It may be assumed that

<sup>1</sup> With some sets of data, curves of  $\log W$  against time would be more appropriate for this purpose.

the 1-day value is a good approximation to the true mean value, and may be used to estimate the magnitude of the error of values based on longer time increments. Table I shows that these errors are small for 4- and 7-day incre-

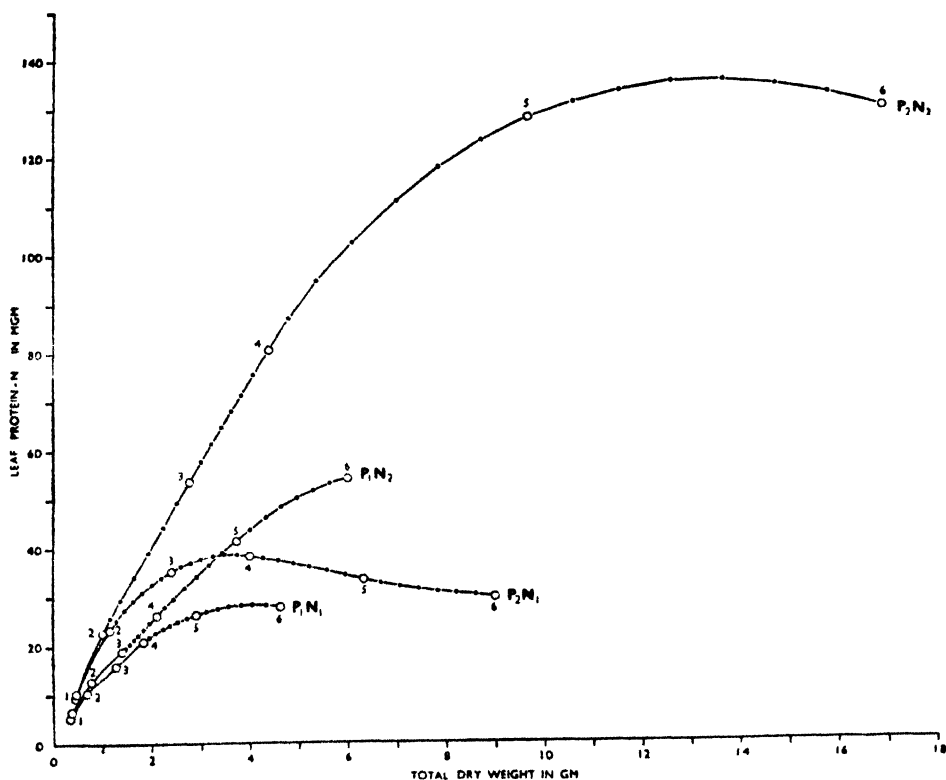


FIG. 1. The  $L_P$ - $W$  relation for *Phalaris tuberosa* L. Manurial treatments and harvest occasions are indicated, and the interpolated values are shown for all but a few early harvest-intervals.

ments, but that they increase rapidly up to 60 per cent. for the case where the  $L_P$ - $W$  relation is assumed to be linear for the whole period. In Table II estimates of the error under discussion are presented for the harvest and

TABLE I

Mean Net Assimilation Rates ( $E_P$ ) for Treatment  $P_2N_1$ , Harvest Interval 1-5

$\Delta t$ , days	1	2	4	7	14	28	56
$E_P$	3.460	3.460	3.468	3.479	3.539	3.837	5.529
Error, %	0	0	0.23	0.55	2.28	10.9	59.8

double-harvest intervals (14 and 28 days respectively) of the whole experiment. An examination of this table in conjunction with Fig. 1 reveals the nature of the factors governing the sign and magnitude of the errors introduced by the direct use of equation IV. As is to be expected, these are negligible

TABLE II  
*Estimated Errors\* in Net Assimilation Rates ( $E_P$ ) calculated direct from Equation IV*

Harvest-interval.	Treatment.			
	$P_1N_1$	$P_1N_2$	$P_2N_1$	$P_2N_2$
A. 14-day intervals				
1-2	+2.5	+4.6	+3.4	+3.5
2-3	+0.7	+0.7	+3.1	+0.2
3-4	+0.2	-0.1	+2.8	-0.2
4-5	+1.6	+0.6	+0.6	+4.7
5-6	+2.8	+2.4	-0.9	+2.0
B. 28-day intervals				
1-3	+7.7	+8.8	+15.5	+7.8
2-4	+0.6	+0.2	+11.5	-0.1
3-5	+5.0	+1.0	+7.1	+7.0
4-6	+7.8	+6.4	-1.5	+15.6

\* Based on the assumption that means of 2-day values of  $E_P$  are correct.

when the  $L_P$ — $W$  relation approaches linearity (e.g.  $P_2N_2$ , interval 2-4); when the relation is concave to the  $W$  axis they are positive; and when it is convex they are negative (e.g.  $P_2N_1$ , interval 5-6). Experience with a wide range of experimental data leads one to suggest that the errors are usually negligible during early growth stages, that large negative errors may occur not only in  $E_P$  but also in direct estimates of  $E_A$  and  $E_W$ . Failure to eliminate such errors<sup>1</sup> is likely to invalidate any attempt to relate  $E$  to factors of the environment.

In the appendix to their paper Briggs, Kidd, and West (1920a) give alternative methods for calculating  $E$ . The first of these is equivalent to equation IV, the second is

$$E = (W_2 - W_1) \div \frac{L_1 + L_2}{2} \quad (V)$$

and assumes that the increase with time in both  $W$  and  $L$  is linear. The method of calculation did not affect the conclusions drawn by Briggs et al., and they used equation V on the ground of its simplicity. During early growth stages, however, equation IV would have yielded the more accurate results as is demonstrated in Table III. The primary data for six early harvests of maize ('Badischer Früh') grown in 1877 (see Briggs et al. 1920, Table III) were used for the calculation of values of  $E_A$  by the graphical method set out above and using daily interpolations; these are compared in Table III with the values calculated from equations IV and V. The superiority of equation IV for these early stages was anticipated from an inspection of the  $L_A$ — $W$  relation for this period.

In concluding this section it should be emphasized that the graphical method of computing  $E$  is open to criticism on the grounds that the drawing

<sup>1</sup> It might be thought that these could be eliminated by reducing the interval between harvests to 7 days or less. In practice, however, this procedure tends to give large experimental errors in successive estimates of increments in  $W$  and hence in the values of  $E$ .



of smooth curves through experimentally determined variables is sure to introduce subjective errors. It is believed, however, that such errors are relatively unimportant.

TABLE III

*Net Assimilation Rates ( $E_A$ ) for 'Badischer Früh' Maize grown in 1877  
(Primary data from Briggs, Kidd, and West, 1920)  
(mg. per sq. cm. per week)*

Harvest-interval.	Method of calculation.				
	Graphical.	Equation IV.		Equation V.	
		$E_A$	Error %*	$E_A$	Error %*
June 12-June 19	6.77	6.92	+2.2	6.08	-10.2
" 19- " 26	5.16	5.26	+1.9	4.83	-6.4
" 26-July 3	5.25	5.29	+0.8	5.03	-4.2
July 3- " 10	3.17	3.18	+0.3	3.13	-1.3
" 10- " 17	5.67	5.85	+3.2	5.65	-0.3

\* Assuming graphical method gives correct value.

In cases where errors from other sources are large, and whenever the  $L-W$  relation approximates to linearity, equation IV will give sufficiently accurate values of  $E$ , and with less calculation.

## ✓ REVIEW OF THE LITERATURE

In Part II of their paper Briggs, Kidd, and West (1920a) point out the general similarity between their curves of relative growth rate and of leaf-area ratio. From this they infer that  $E_A$  is more or less constant throughout the life-cycle of the plant, and set out to test this inference by computing values of  $E_A$  from Kreuzler's comprehensive data for maize. In discussing the ideal form of the curve for  $E_A$ , they point out that the known increase in the assimilatory activity of seedling leaves would produce an initial rise in the rate. Furthermore, they show that respiratory losses in all parts of the plant when considered in relation to leaf area should accentuate the initial rise, and subsequently produce in  $E_A$  a fall with time. Both of these factors belong to the complex of 'internal factors' which on theoretical grounds could influence net assimilation rates. Although only the initial rise could be demonstrated from the analysis of Kreuzler's data, the possible importance of the respiration factor should not be overlooked as a determinant of  $E$  during late stages of growth.

In their treatment of the relative growth-rate data of these experiments, Briggs et al. (1920) also demonstrate subsidiary maxima which coincide with the time of appearance of male and female flowers; they are inclined to interpret them in terms of increased respiratory activity during flower development. If this interpretation be correct these phenomena would add to the complexity of the 'internal factor'.

Briggs et al. also correlated the early and more reliable values of  $E_A$  with certain factors of the environment and found that the rate was governed more by mean temperature than by the hours of sunshine; the tentative explanation advanced is that temperature may act via an effect on stomatal movement, or 'that it is growth (i.e. utilization of assimilated material) governed by temperature which controls assimilation'. The use by these workers of the method of correlation is open to the criticism that no allowance is made for possible time trends in the dependent variable;<sup>1</sup> nevertheless, it must be admitted that an inspection of Figs. 3–6 of their paper reveals remarkably similar time trends in  $E_A$  and in mean temperature.

So many workers have considered that the experiments of Gregory (1926) with barley have established that  $E_A$  is independent of time prior to the attainment of maximum leaf area, that it seemed necessary to examine the grounds for his conclusion in some detail. Gregory conducted five growth experiments in four successive seasons, and in two cases the plants received a fourfold dressing of nitrogen. From each experiment, the six or seven weekly values of  $E_A$  prior to the time of maximum leaf area were pooled for the calculation of partial correlation coefficients with average day temperature, average night temperature, and with total radiation. The validity of this procedure depends on the assumption that  $E_A$  is independent both of time and manurial treatment, but Gregory produced no evidence to support this assumption. From the regression equation for his set of partial correlation coefficients, Gregory concluded that 'over 80 per cent. of the variation in assimilation rate is accounted for by change in climatic conditions' (p. 10), and he found it remarkable that the time factor and the quantity of nitrogen added as manure should have such a small effect.

With reference to the effect of nitrogen, it has been shown by Crowther (1934) and by Ballard and Petrie (1936) that nitrogen supply, over a certain range, has a negligible effect on  $E_W$  during early stages of growth. Similarly the work of Gregory and Richards (1929) and of Chinoy (vide Gregory, 1937) on the effect of nitrogen deficiency on carbon assimilation, expressed per unit area, indicates that  $E_A$  also may be unaffected.

As for the time factor, it is not yet established beyond question that  $E_A$  is ever constant prior to maximum leaf area, and the work of Gregory and Richards (1929), though of limited value in this respect, indicates a decline in the assimilation rates of successive mature leaves of barley.

Assuming there was a time factor governing Gregory's values of  $E_A$ , its effect would become positively or negatively associated with any general time trend in one or more of the factors of the environment, and to that extent would be included in the 80 per cent. of the variation attributed to these factors. That such an association could have occurred is indicated by the fact that four of the five sets of data presented in Fig. 1 of Gregory's paper show downward trends with time.

<sup>1</sup> This point will be elaborated below in discussing the work of Gregory (1926). It seems that 'internal factors' were only minor determinants of  $E_A$  during the early growth of these maize plants.

Crowther (1934) gives values of  $E_w$  for the cotton plant, but these refer only to the aerial parts. He found the rate to be unaffected by either water or nitrogen supply up to the time of maximum leaf weight. The water treatments (rates of irrigation) were evidently such that differential wilting of the leaves did not occur to any extent, hence the rate of carbon assimilation was little affected. The considerable fluctuations of  $E_w$  with time are attributed by Crowther to variations in climatic conditions; there is no reason, however, to exclude the possibility that internal factors operated concurrently (vide Heath and Gregory, 1938). In a later publication Crowther (1937) gives a generalized mean curve for  $E_w$  (Fig. 14, p. 37) based on 10 experiments with cotton. This curve shows a maximum at approximately 70 days after sowing, and thereafter declines until, at the time of maximum leaf weight,  $E_w$  is little more than one-third of its maximum rate. On the basis of Gregory's conclusions and 'assuming that leaf weights reflect differences similar to those of leaf areas', Crowther says that this decline must be due to the progressive shading of the leaves as the plants increase in size. While this explanation is plausible, it may be significant that the curves for mean nitrogen content of the leaves (Fig. 20, p. 48) are similar in form; so similar, in fact, that values of  $E_N$  would be almost constant. In the absence, therefore, of any definite knowledge that light intensity as affected by shading was limiting the rate of photosynthesis under the conditions of the experiment, one may not exclude the possibility that leaf-nitrogen, or some better index of the cytoplasm content of the leaves, was causally related to the observed trend in  $E_w$ .

Ballard and Petrie (1936) point out that, subsequent to the initial rise in  $E$  predicted by Briggs et al. (1920a), both  $E_A$  and  $E_w$  would fall because of the decrease in the assimilatory activity of the individual leaves as they grew older, and the increasing mean age of the leaves as a whole. Their data for  $E_w$  with wheat and Sudan grass amply bear this out. In both experiments a wide range of nitrogen treatments was included, and for Sudan grass  $E_w$  fell continuously from the first observation. For wheat,  $E_w$  values were limited to the latter half of the life-cycle, and also fell with time. As was indicated above, nitrogen supply had no effect on  $E_w$  during the early stages of growth of Sudan grass, but the rate was significantly depressed by nitrogen deficiency in the wheat experiment. Ballard and Petrie regarded it as very improbable that the general time trends in  $E_w$  were due to environmental factors.

Williams (1936) presented  $E_w$  values for two experiments with oats as affected by the varying supply of phosphorus. In the second experiment no consistent time trends were found in  $E_w$  for the first 6 weeks of growth, but there were significant effects of treatment which implied the existence of internal factors governing the rate. After this period, and in both experiments,  $E_w$  fell rapidly with the two higher phosphorus treatments, but less rapidly with low phosphorus supply; treatment relations with respect to  $E_w$  were thereby reversed. The data of the second experiment are incorporated in Fig. 9 of this paper.

From his experiments with cotton grown in South Africa, Heath (1937,

1937a) concludes that  $E_w$  shows no general rise or fall up to the time of flowering, and claims that this conclusion confirms the findings of Gregory (1926). Quite apart from the admitted accuracy of Heath's *statistical* use of the words *not proved* (vide Williams, 1937, Heath, 1938) in relation to the absence of time trend in  $E_w$ , the mere absence of such trend in the *original* data cannot logically be used to support the claim that  $E$  is independent of time. It has been shown above that Gregory's *original*  $E_A$  values show some pronounced downward trends with time, and that the proposition concerning the constancy of  $E_A$  in a constant environment is as yet unproven for the experiments with barley.

The inevitable omission of root weights from Heath's data for field-grown cotton is a complicating factor. Quite apart from its possible effect upon the time trend in  $E_w$ , this factor could account for the significant effect of cultivation treatment on the rate. If not, then the differences in  $E_w$  imply the operation of internal factors, whose nature is not apparent from the data.

Watson and Baptiste (1938) studied the growth of sugar-beet and mangold as affected by time of sowing; the six sowing dates and the subsequent sampling occasions were at 14-day intervals. In Fig. 13 of their paper values of  $R$  and  $E_A$  are presented for ten of these harvest-intervals, the values being means for all sowing dates. Estimates of error were derived from deviations from linear regressions on sowing-date (treatments not being replicated). From an examination of linear regression coefficients<sup>1</sup> of  $R$  on sowing-date the authors conclude that this quantity is dependent both on internal factors of age and on external factors. Evidence is given to show that the age effect is at least in part accounted for by the time trend in the ratio of leaf area to total plant weight. The values for  $E_A$  were examined in the same way, and since no significant relation to sowing-date could be found, it is concluded that the fall with time in this quantity can scarcely be attributed to internal factors. An attempt was then made to establish a relation between  $E_A$  and certain factors of the environment, but no correlation with radiation or temperature could be demonstrated apart from the linear fall in all three variables.

Watson and Baptiste therefore conclude that the fall of  $E_A$  with time was caused by a fall in radiation or temperature, and not by a change in internal factors of age. This argument overlooks the possibility that the test of significance applied to the linear regression coefficients on sowing-date may be insensitive. Indeed, the individual values of  $E_A$  contributing to the mean values of Fig. 13 would need to have absurdly wide ranges before significant effects of sowing-date could be demonstrated.<sup>2</sup> This being the case, it should be noted that the first five coefficients for sugar-beet, and with one exception, the first six for mangold, are positive, thus suggesting an effect of age during early growth. The evidence, though not conclusive, indicates that both

<sup>1</sup> These are derived in two ways: (a) from the six values at each sampling time, and (b) from the sets of six values for plants of equal age.

<sup>2</sup> Using the significant differences given in Fig. 14 it can be shown that these ranges have minima of 0.96 and 1.42 gm. per sq. dm. per week for sugar-beet and mangold respectively. Thus for sugar-beet at harvest interval 5-6, values of 0.15 and 1.05 units for plants of the first and sixth sowing dates could not be regarded as indicative of the existence of internal factors of age.

external and internal factors contribute to the observed time trend in  $E_A$  as well as to that in  $R$ .

Williams (1939) considered that the progressive decrease in the cytoplasm content of the leaves would be an important factor contributing to a general decline in  $E_W$  with time, and using protein nitrogen as a measure of this content, he presented  $E_P$  values for four of the experiments discussed above (Ballard and Petrie, 1936; Williams, 1936). In general, the time trends in  $E_P$  were quite closely related to those in mean maximum temperature; a relation which was not apparent between  $E_W$  and temperature. In plants of low nitrogen status<sup>1</sup> it was inferred that, throughout a considerable portion of the growing period,  $E_P$  would be constant in a constant environment. Although treatment effects were absent during very early growth,  $E_P$  later became depressed in plants of high nitrogen status, the depression being attributed to the accumulation of storage proteins in the leaves or to a decreased effectivity of the cytoplasm as a whole.

Petrie, Watson, and Ward (1939) studied the effects of phosphorus supply and topping on the tobacco plant and included data for  $E_W$  and  $E_A$ . Values for two of their treatments are presented in Fig. 2, together with values of  $E_N$  computed from the nitrogen data given by Watson and Petrie (1940). It is admitted that the initial values may be low because of a temporary setback to the plants following transplantation. After the second harvest-interval, however, all values of  $E$  fall rapidly with time,  $E_W$  most and  $E_N$  least rapidly. Since the trends in  $E_N$  show no obvious relation to those in the climate indices available, it must be concluded that leaf nitrogen is in this case an inadequate index of the 'internal factor' for growth, or that some unmeasured factor of the environment, such as the progressive shading of the leaves, is responsible for the fall in  $E_N$ .

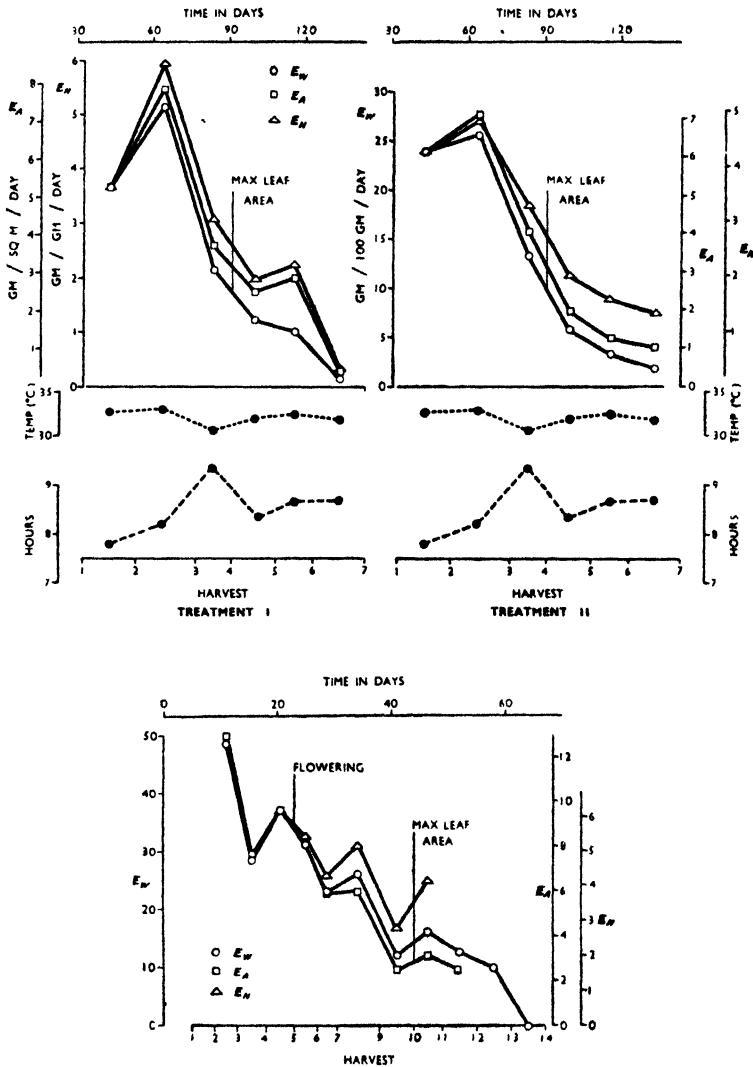
In their studies of the effects of artificial drought applied to flax and linseed after flowering, Tiver (1942) and Tiver and Williams (1943) present growth analyses which include values for both  $E_W$  and  $E_P$ . Both rates were greatly depressed by treatment, as would be expected in plants whose leaves wilted when the soil-moisture level approached the permanent wilting percentage. In the control series the time trends in  $E_P$  were more clearly related to the environment than were those in  $E_W$ ; even so, there were certain anomalies in the  $E_P$  values. Evidence was then adduced to show that the nitrogen status of both plants was high, and it was shown that  $E_P$  may sometimes be used as an inverse index of the changing status within the plant.

Petrie and Arthur (1943) present values of  $E_W$ ,  $E_A$ , and  $E_P$  for a further experiment with tobacco; the data, in this case, are more complete for early growth stages, and the treatments include both permanent and temporary drought. It is evident that the leaf attributes,  $L_W$ ,  $L_A$ , and  $L_P$ , for tobacco vary greatly in their relations one with another,<sup>2</sup> and Wood, the compiler of the

<sup>1</sup> Plants which had received low supplies of nitrogen or high supplies of phosphorus.

<sup>2</sup> See Petrie, Watson, and Ward (1939) for a full treatment of the  $L_W-L_A$  relation in tobacco leaves.

paper, clearly establishes his preference for expressing  $E$  on a leaf-protein basis. Three factors may contribute to the lack of dependence of  $E_P$  upon temperature. Thus other factors of the environment may have been con-



FIGS. 2 and 3. Fig. 2. Net assimilation rates ( $E_w$ ,  $E_A$ , and  $E_N$ ) for tobacco (Petrie, Watson, and Ward, 1939). Treatments are 0.1 and 0.3 gm. P (as  $\text{NaH}_2\text{PO}_4$ ) per pot respectively, and the climate indices are temperature and hours of bright sunlight per day. Fig. 3. Net assimilation rates ( $E_w$ ,  $E_A$ , and  $E_N$ ) calculated from the data of Maiwald (1930) for buckwheat. The scales are adjusted so that all three rates have the same ordinate for harvest-interval 4-5.

cerned; there may have been concurrent changes in the nitrogen status of the plants (vide Tiver and Williams, 1943); and finally, the relation to temperature may have been upset by the incidence of transplantation between harvests

3 and 4. The plants would undoubtedly suffer a check as a result of this operation, and the trend in  $E$  would be further complicated by the fact that such plants were selected for vigour and uniformity. The effects of drought on  $E_P$  were similar to those reported by Tiver (1942) and by Tiver and Williams (1943).

An interesting application of the methods of growth analysis here considered is that made by Grieve (1943) in his study of the effects of spotted wilt on the growth of tomato. Grieve demonstrated that the depression of  $R$  in diseased plants is attributable to an effect on  $E_A$  and not to any effect on the leaf area ratio. He also showed that the tomato spotted wilt virus depresses starch formation and delays its hydrolysis. It is concluded that the effect of the virus on growth in dry weight is by partial destruction and reduction in efficiency of the assimilating tissue.

There are in the literature certain papers in which the quantitative analysis of plant growth *per se* is not the concern of the experimenter, but which contain sets of data from which values of  $E$  may be determined. Some of these papers will now receive brief consideration. The relevant numerical data have been tabulated as part of a supplement to this paper.

Maiwald (1930) gives data for buckwheat (*Fagopyrum esculentum* Moench) from which values of  $E_H$ ,  $E_A$ , and  $E_N$  may be calculated (see Fig. 3). To facilitate comparison of the time trends, the scales were adjusted so that all three rates had the same ordinate for harvest-interval 4-5. Flowering begins early in buckwheat, and maximum leaf area is attained late in the life-cycle. Apart from minor fluctuations,  $E_H$  falls continuously, and at the time of maximum leaf area is little more than one-quarter of its initial value. An unusual feature is that  $E_A$  falls even more sharply with time than does  $E_H$ ; this follows from the fact, demonstrated by Maiwald himself, that the dry weight per unit area of leaf decreases with time. The leaf-nitrogen data are available only for harvests 4-11 inclusive, but for this period  $E_N$  falls less rapidly than does  $E_H$ . That the climatic factors were in part responsible for the trends in all three estimates of  $E$  is rendered probable by the statement (see p. 50) that the weather was favourable from day 27 to day 38, but wet and cold from day 38 to day 49.

Net assimilation rates ( $E_H$  and  $E_N$ ) were calculated from the primary data of Wagner (1932 and 1932a) for oats and sugar beet, of Knowles, Watkin, and Hendry (1934) for sugar-beet, and of Lewis and Marmoy (1939) for tomato. For a number of reasons, including the high variability of the resulting values of  $E$ , these data are not entirely satisfactory for the present purpose; however, it is worthy of note that the time trends in  $E$  are similar in all cases, and that the downward trend in  $E_N$  is less steep than that in  $E_H$ . In like manner, weekly values of  $E_A$  may be calculated from the data of Ashby (1937) for tomato. In all these cases it would be unwise to infer the existence of an 'internal factor' governing the rate because adequate climatic data are not available.

In a study of the growth of *Solanum nodiflorum* Jacq. (= *S. nigrum* L.) Larsen (1942) compares the directly-estimated with the calculated production of dry matter, the latter being derived from measurements of carbon assimilation and respiration. The similarity of the resulting curves lends support to the assumption that change of weight in plants is primarily determined by

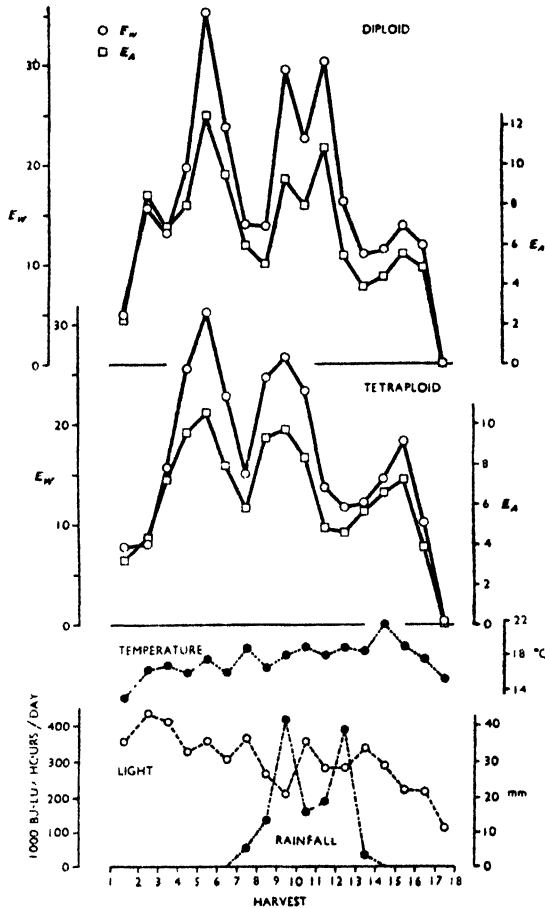


FIG. 4. Net assimilation rates ( $E_W$  and  $E_A$ ) calculated from the data of Larsen (1942) for diploid and tetraploid plants of *Solanum nigrum* L.  $E_W$  is expressed in gm. per 100 gm. per day and  $E_A$  in gm. per sq. m. per day. Flowering commenced at harvest 6 and maximum leaf area and leaf weight occurred at harvest 12 in each case.

these two physiological functions. In effect, Larsen determined successive values of  $E_A$  by direct measurement, and showed that the growth curve could be calculated therefrom. Unfortunately neither these values nor their indirectly determined equivalents were published by Larsen. The values of  $E_A$  and  $E_W$  shown in Fig. 4 of the present paper were calculated from the  $W$  values of Table I and the  $L_A$  and  $L_W$  values of Figs. 1 and 2 of the original paper; the climatic indices were taken from Fig. 4. Maximum  $L_A$  and  $L_W$



occurred at harvest 12 for both diploid and tetraploid plants, and the inflorescences were first separated at harvest 6. Harvest 1 coincided with transplantation into the field plots; this fact, together with the dryness of the period immediately following, suggests that some of the values of  $E$  prior to harvest 8 may have been sub-optimal. The general time trends in both  $E_W$  and  $E_A$  between harvests 4 and 16, however, are downward, and might be attributed to the general decrease in the amount of light per day. On the other hand, the mean temperatures rise slightly over the same period, and there is no obvious correlation between the successive values of  $E$  and those of the climatic indices. The general similarity of the curves for diploid and tetraploid plants could be attributed to internal factors specific to this plant or to environmental factors not measured by Larsen.

There remain a few papers in which the concept of net assimilation rate is used, but in which conditions, either external or internal, are more rigorously controlled in order to study the effects of specific variables. Thus Portsmouth (1937) studied the effects of three types of illumination (continuous and two rates of intermittent light) on cucumber plants grown at constant temperature and humidity. The reduction in  $E_A$  with intermittent light of 1-minute alternations was traceable, at least in part, to stomatal closure.

Bolas, Melville, and Selman (1938) used a measure of carbon assimilation which is essentially an estimate of  $E_W$  over the 7-hour period of each experiment. Using a 'paired-plant' technique these workers attempt to evaluate the response of tomato seedlings (each having 7–8 expanded leaves) to the conditions obtaining throughout the year in normal glass-house practice. For seven of their experiments they also give estimates of  $E_A$ . Their selection of an arbitrary stage in the plants' development is an attempt to 'control' the 'internal factor' for growth, and its partial success is evident from the statistical analysis of the data. Over the ranges of light intensity and temperature encountered, the correlation of  $E_W$  with temperature was positive and significant, but with light it was insignificant (cf. Williams, 1939). Some of the admitted variability introduced by the previous history of the experimental plants might have been eliminated if assimilation had been measured on a protein rather than a leaf-weight basis. However, such a refinement of technique would not be justified if the nitrogen status of the plants were unduly high (vide supra).

The work of Ashby and Oxley (1935) and of White (1937, 1939) with *Lemna* has included studies of the effects on  $E_A$  of the following interacting factors—light intensity and temperature, nitrogen and light intensity, and potassium and light intensity. White (1936) has also drawn attention to strain differences in *Lemna* with respect to  $E_A$ . The relationships revealed by these experiments are quite complex in spite of the nature of the experimental material and, while it is true that the response of a higher plant might be very different from that of *Lemna*, this work should prove of great value in the interpretation of similar experiments with higher plants. In the experiments

in which the nutrient levels were varied, White (1937, 1939) also obtained estimates of the protein contents of the fronds. He was able to demonstrate that at a given light intensity,  $E_A$  and protein content were positively correlated.

## PRESENTATION OF NEW DATA

### 1. *Introductory*

The growth experiment to be described was limited to the vegetative phase of growth of the perennial pasture grass, *Phalaris tuberosa* L. The experiment terminated approximately 8 weeks before anthesis and 4 weeks before exertion of the inflorescences was expected to commence. No attempt was made to determine the time of 'first differentiation of flowers' (vide Heath, 1938), but with all treatments, total leaf weights were increasing throughout the experiment. Leaf area was not measured, but from inspection it was clear that the maxima did not occur prior to final harvest.

A better demonstration of the relation between  $E_P$  and factors of the environment could have been achieved by a restriction to one manurial treatment by which growth would be limited by nitrogen supply (Williams, 1939); but it was desired to demonstrate the limitations as well as the advantages of expressing  $E$  on a protein-nitrogen basis. The experiment was designed so that it would be possible to eliminate the effects of position in the glasshouse from the quantities measured, and to obtain true replicates for the derived quantities  $R$  and  $E$ .

The primary purpose of the experiment was to see whether protein nitrogen was more generally satisfactory than dry weight as a basis for expressing net assimilation rates. Although the nutrient effects on attributes other than  $E$  are of considerable interest, discussion of these effects will be strictly limited.

### 2. *Experimental Procedure*

On April 30, 1937 (day 0), seeds of a pure line of the perennial pasture grass, *Phalaris tuberosa* L., were sown in pots containing 15 K. of water-washed sand. The experiment was conducted in a glasshouse, and the water content of the sand was maintained by watering to 67 per cent. of its saturation capacity.

With the exception of the iron and manganese, which were added on May 25, all nutrients were added prior to the date of sowing. All pots received the following quantities (gm. per pot) of dissolved mineral salts:

KCl, 4.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0;  $\text{Na}_2\text{SO}_4$ , 1.0;  $\text{FeCl}_3$ , 0.1;  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 0.002.

Nitrogen and phosphorus were added as calcium nitrate and calcium acid phosphate respectively, there being two levels of each in all combinations. Calcium was equalized for all treatments by the addition of appropriate

amounts of calcium chloride. The amounts per pot of the three elements which varied with treatment were as follows:

Treatment.	N.	P.	Cl.
$P_1N_1$	0.4 gm.	0.04 gm.	3.15 gm.
$P_1N_2$	1.6 „	0.04 „	2.05 „
$P_2N_1$	0.4 „	0.24 „	3.06 „
$P_2N_2$	1.6 „	0.24 „	1.97 „

The choice of these levels of nitrogen and phosphorus was based on previous experience, and in the expectation that final yields would be

$$P_1N_1 < P_1N_2 = P_2N_1 < P_2N_2.$$

On day 21 the seedlings were thinned to 5 uniform and evenly spaced plants per pot. The six harvests of the experiment were taken on days 69, 83, 97, 111, 125, and 139 respectively, and at each harvest one pot per treatment was selected at random from each of 6 blocks.

Leaf separations were made by cutting at the ligule, but in the case of the youngest leaf of each tiller only that portion which had protruded from the sheath of its predecessor was taken. The 'stem' fraction included true stems, the leaf sheaths, and young unexposed leaves. Roots were severed as close to the stems as practicable, and later sieved under water until practically free from sand. The fresh weights of the leaves were determined immediately after separation, and the leaves dried rapidly under forced draught at 80° C. The more fleshy stems and the root systems were dried at 100° C. As the root systems were still grossly contaminated with sand, they were subsequently incinerated, the ash constituents dissolved out, and the residues weighed and used as correction figures for the crude dry weights.

The method used for estimating protein nitrogen in the dry leaf material is described by Petrie (1937) and consists essentially of a brief aqueous extraction of soluble nitrogen compounds at 100° C., followed by a precipitation of the dissolved proteins with tannic acid. After filtration, protein nitrogen was estimated by the Kjeldahl procedure.

The mean day and night temperatures presented in Fig. 7 are derived from thermograph records made in the glasshouse and are obtained by a process of graphical integration. The hours of bright sunlight were recorded nearby at the Waite Institute meteorological station.

### 3. *Presentation and Statistical Treatment*

Only a limited amount of the primary data is presented in tabular form, and the derived quantities *R* and *E* are presented graphically in Figs. 4–6. A full set of the tabular data may be seen at the Natural History Museum, South Kensington, London, and a limited number of copies are available on application to the Waite Institute, Adelaide, South Australia.

Statistical treatment of the data was by the analysis of variance, and was quite straightforward except for tiller-number and dry-weight data. In these cases a logarithmic transformation was required to eliminate the correlation between the intra-class variance and the class mean; the mean logarithms are

given in *italics* in Tables IV and V. Where significant differences are mentioned in the test, these are at the 1 per cent. level of significance unless otherwise stated.

The replication was 6 for all sets of data other than those for  $E_p$ . Protein analyses of the leaves from each pot were scarcely justified in the light of the results for  $E_w$ ; hence the leaf material was bulked for each harvest class. The statistical analyses for  $E_p$  (see supplement) were conducted as in a factorial experiment ( $2 \times 2 \times 5$  and  $2 \times 2 \times 4$  according as the time-interval taken was 14 or 28 days in length), and since the second-order interaction and two of the first-order interactions ( $P \times T$  and  $N \times T$ ) were of similar magnitude, these were pooled to give an estimate of error variance.

#### 4. Primary Data

##### (a) Leaf and tiller production

Phalaris is a small-seeded grass, and since the embryo is also small, it might be expected that early growth and development would be slow by comparison with large-seeded cereals (vide Ashby, 1937). The retardation in growth (as measured by dry weight production) was such that the first harvest was delayed until day 69; prior to that time there would have been insufficient material for the projected chemical analyses. Table IV gives the mean number

TABLE IV

*Mean Number of Leaves on the Primary Shoot and Mean number of Tillers per Plant*

*Figures in Italics are Mean Logarithms of these Values*

Days from sowing.	Harvest.	Treatment							
		$P_1 N_1$		$P_1 N_2$		$P_2 N_1$		$P_2 N_2$	
		Leaves on primary shoot							
54	—	5.39	—	5.51	—	5.72	—	5.83	—
		Tillers per plant							
54	—	1.19	—	1.32	—	1.70	—	1.84	—
69	1	3.3	0.52	3.4	0.53	4.3	0.63	4.4	0.64
83	2	5.2	0.71	5.7	0.75	9.0	0.95	8.5	0.92
97	3	7.5	0.88	6.5	0.81	11.7	1.07	13.2	1.12
111	4	8.7	0.94	8.8	0.94	13.5	1.13	16.2	1.21
125	5	9.5	0.97	11.8	1.07	13.9	1.14	22.3	1.35
139	6	10.4	1.01	12.5	1.10	13.5	1.13	23.7	1.37

S.E. for means of 6 = 0.021

Sig. diff. (1%) for comparison of means of 6 = 0.080

of leaves on the primary shoot on day 54 and the number of tillers per plant on this day and at each harvest. At all stages and at both levels of nitrogen supply, tiller number was increased by increased phosphorous supply. The effect of nitrogen supply on tiller number was not apparent until the later stages of growth, and was then greater at the higher level of phosphorus. The

single set of observations on leaf number likewise suggests that early meristematic activity is stimulated more by phosphorus than by nitrogen.

(b) *Dry weight*

The data for total plant and for leaves are presented in Table V; those for the stems and roots are tabulated in the supplement. When plotted on a logarithmic scale the total-plant data suggest a fairly close approximation to linearity within each treatment, but as the data for *R* will show, this approximation is more apparent than real; the effects of both time and treatment on *R* are considerable. Thus these data do not conform at all strictly to the compound interest law of Blackman (1919).

TABLE V

*Dry Weights (gm. per plant). Figures in Italics are Mean Logarithms of the Values*

Days from sowing.	Harvest.	Treatment							
		P <sub>1</sub> N <sub>1</sub>		P <sub>1</sub> N <sub>2</sub>		P <sub>2</sub> N <sub>1</sub>		P <sub>2</sub> N <sub>2</sub>	
TOTAL PLANT									
69	1	0.341	0.529	0.386	0.583	0.445	0.639	0.465	0.665
83	2	0.689	0.829	0.790	0.892	1.159	1.056	1.003	0.993
97	3	1.28	1.107	1.40	1.141	2.41	1.375	2.81	1.448
111	4	1.83	1.261	2.11	1.317	4.02	1.599	4.41	1.632
125	5	2.91	1.459	3.75	1.572	6.33	1.799	9.65	1.978
139	6	4.63	1.662	6.04	1.781	9.01	1.954	16.83	2.225
S.E. for means of 6 = 0.0240									
Sig. diff. (1%) for comparison of means of 6 = 0.0932									
LEAVES									
69	1	0.130	0.108	0.158	0.195	0.204	0.302	0.219	0.340
83	2	0.253	0.394	0.308	0.483	0.517	0.708	0.491	0.684
97	3	0.445	0.647	0.520	0.711	0.944	0.969	1.230	1.090
111	4	0.620	0.789	0.748	0.868	1.459	1.161	2.052	1.301
125	5	0.93	0.964	1.32	1.121	2.17	1.336	3.98	1.595
139	6	1.57	1.191	2.04	1.309	2.97	1.472	6.10	1.785
S.E. for means of 6 = 0.0218									
Sig. diff. (1%) for comparison of means of 6 = 0.0850									

The initial stimulus by phosphorus to meristematic activity is reflected in the dry-weight data for leaves, stems, and for total plant, but not in those for roots. The latter can be attributed to the established effect of this nutrient on root-weight ratios (Williams, 1936). At both levels of nitrogen the response to phosphorus increases with time and becomes greatest at the higher level (N<sub>2</sub>). In like manner the late response of nitrogen is very much more pronounced at the higher level of phosphorus; this applies, however, more to the data for leaves and stems than to those for roots. All of these comparisons are based, of necessity, on the mean logarithms of the data.

The leaf, stem, and root weight ratio data are presented in the supplement and will not be discussed in any detail here. However, it may be noted that the leaf weight ratios show a fairly uniform downward trend with time; that phosphorus increases the initial value of the ratio at both levels of nitrogen;

but that, at the lower level, this effect disappears because of the more rapid decline in the ratio with treatment  $P_2 N_1$ . The increase with increased nitrogen supply is small but fairly consistent at the lower level of phosphorus supply, but is large for harvests 4 and 5 at the higher level of supply.

In general, it may be said that the effects of time and treatment on the weight ratios of plant parts conform to the findings of Ballard and Petrie (1936) and Williams (1936).

### (c) *Protein Nitrogen*

The relative and absolute data for leaf water and leaf protein-nitrogen may also be found in the supplement. The absolute protein-nitrogen values were used for the calculation of  $E_P$  (see also Fig. 1), but comment here will be restricted to the relative-protein data. Thus at harvest 1, nitrogen supply had no effect, whereas phosphorus supply increased the protein-nitrogen content from 4.1 to 4.6 per cent. This, together with the still greater increases in absolute protein-nitrogen, suggests that phosphorus is directly concerned in protein synthesis (cf. Richards and Templeman, 1936). The effects of the lower nitrogen supply on protein contents is expressed in their more rapid downward trends with time. At the lower phosphorus level this effect is not apparent until harvest 5, but at the higher level it is quite pronounced at harvest 3 and the fall is rapid for the rest of the experiment.

These time-treatment interactions on protein content are similar to those found by Petrie (1937) and Williams (1938) with varying supplies of nitrogen and phosphorus respectively. They also conform to the general picture obtained by Mathur (vide Gregory, 1937) for nitrogen content in barley as affected by varying supplies of nitrogen and potassium; both sets of data may be interpreted largely in terms of the amount of growth made.

## 5. *Growth Analysis (Derived Data)*

### (a) *General*

The quantities  $R$  and  $E$  are subject to relatively large experimental errors. This may be illustrated by a comparison of some of the results of the analyses of variance for the primary data with those for the derived quantities. Thus for  $W$ , 26 of the 36 possible treatment comparisons were significant, and for  $L_W$ , no fewer than 32 of 36 such comparisons were significant; for  $R$ , on the other hand, only 5 out of 30 were significant, and for  $E_W$ , not one of 30 comparisons reached the 1 per cent. level of significance. It should be understood that these statements apply only to individual means of 6, and for  $R$  and  $E$ , only to the values for 14-day intervals (see upper left-hand corners of Figs. 5 and 6). Even the five significant differences for  $R^1$  must be accepted with caution because the second-order interaction ( $P \times N \times \text{time}$ ) was insignificant, and because the data for  $P_2 N_2$  exhibit pronounced fluctuations which suggest one or more aberrant dry weight values (vide Williams, 1939). For these

<sup>1</sup> At harvest-interval 2-3,  $P_2 N_2$  is  $>$  all other treatments, and at 4-5,  $P_2 N_2$  is  $>$   $P_1 N_1$  and  $P_2 N_1$ .

reasons the significant difference appropriate to means of 6 (14-day interval) was omitted in Fig. 5.

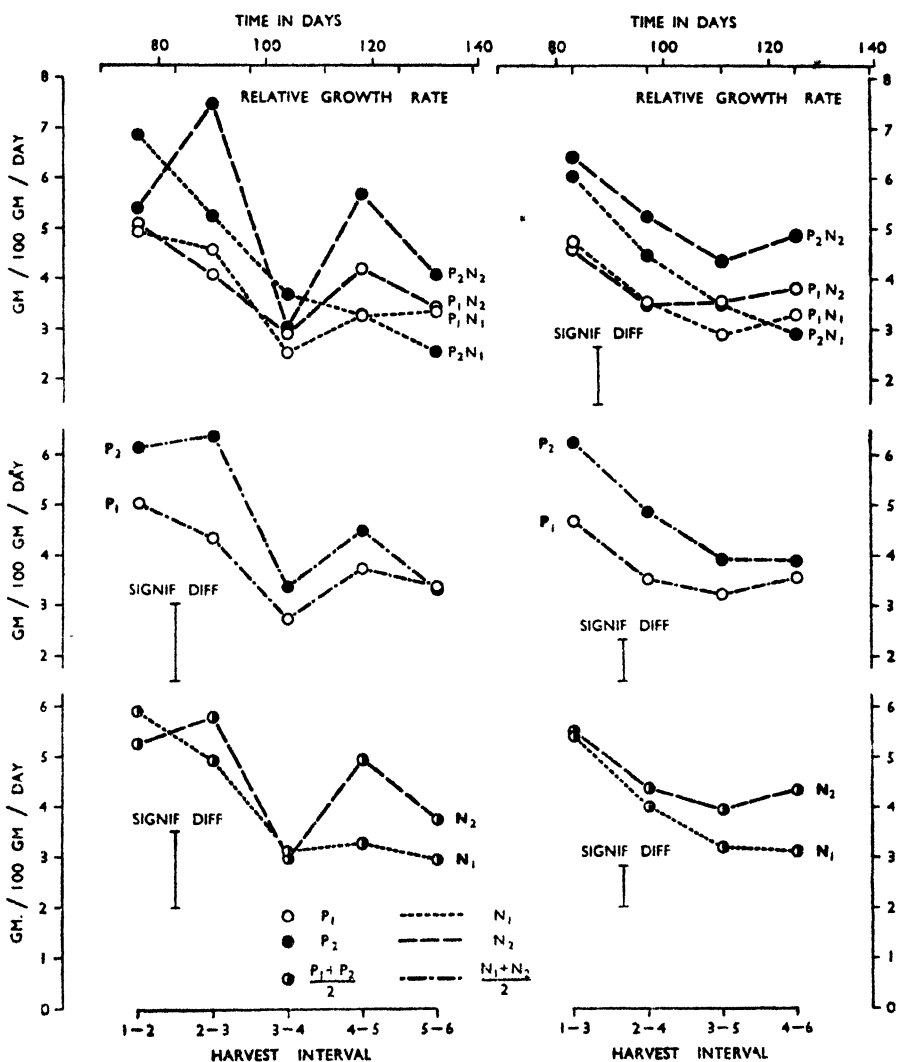


FIG. 5. Relative growth rates ( $R$ ) for *Phalaris tuberosa* L. These are for 14-day (left) and 28-day intervals (right) and means appropriate to the first-order interactions are plotted below the sets of harvest class means. Significant differences (1 per cent.) are shown as vertical lines.

The error under discussion decreases with an increase in time interval, so that for  $R$  the standard error with 28-day intervals is little more than half its value with 14-day intervals. With the former interval, 9 out of 24 possible treatment comparisons are significant, and although the second-order interaction is again insignificant, the treatment-time interactions are much more consistent (compare the two sets of data in the upper part of Fig. 5); for this

reason the 9 significant differences mentioned may be accepted with confidence. Further information about time trends and general effects of variation in nitrogen and phosphorus supply was obtained by examining the first-order interactions (e.g.  $N \times \text{time}$ ).

When the primary purpose of a growth experiment is to ascertain the form of the relation between net assimilation rate and individual factors of the environment, the advantage of long time-intervals is offset by the smoothing out of natural fluctuations in all the variables. This tendency is illustrated in the data of  $E_w$  and  $E_p$  of the present experiment: with 28-day intervals only general time trends are apparent, but with 14-day intervals the fluctuations in  $E$  are sufficiently consistent to suggest that they are determined in part by the environment. Even so, the data are too variable and too inadequate for the purpose of establishing such relations on a statistical basis.

### (b) *Relative Growth Rate*

Wood (vide Petrie and Arthur, 1943) discusses the merits of growth rate  $dW/dt$  and of relative growth rate  $(1/W)(dW/dt)$  for the purpose of growth analysis. The points in favour of the latter are clearly stated, but for treatment comparisons Wood prefers the former. The example given (*loc. cit.*, p. 198), however, could also be used in support of  $R$  as the appropriate measure of growth; for although  $R$  becomes approximately constant for the low- and high-water series it is evident that the initial difference in dry weight implies an initial difference in  $R$  for harvest-interval 3-4 ( $R = 0.108$  and  $0.146$  for low- and high-water respectively). The two measures are complementary in the same sense as are the relative and absolute contents of nitrogen in the leaves of differently treated plants. Only  $R$  will be considered here, because the detailed analysis of treatment effects is beyond the scope of this paper.

The data for both 14- and 28-day intervals are presented in Fig. 5, means appropriate to the first-order interactions being plotted below the respective sets of harvest-class means. With the shorter time-interval the picture is somewhat erratic, and the second-order interaction is insignificant. The first-order interactions,  $P \times \text{time}$  and  $N \times \text{time}$ , are significant at the 1 per cent. and 5 per cent. points respectively; from these a significant general effect of phosphorus is demonstrable for harvest-interval 2-3, and one of nitrogen for interval 4-5. In neither case do these effects greatly exceed the significant difference figure, but each gains support from one or more adjacent but insignificant effects of the nutrient concerned. In spite of pronounced fluctuations, these 14-day values indicate a general fall with time.

When the longer time-interval is used the time trends and treatment effects are far more consistent. The general effect of phosphorus is significant for intervals 1-3 and 2-4, and that of nitrogen for interval 4-6. This is another way of stating that phosphorus stimulates early growth and that the effect of nitrogen tends to be delayed. The general effects of treatment on  $R$  may be analysed by studying the effect of each at the two levels of the other. Thus the early effects of phosphorus is much the same at both levels of nitrogen, but the



late effect of nitrogen is significant at the high but not at the low level of phosphorus. A downward trend with time is evident in all cases, but it varies in form and extent from treatment to treatment.

(c) Net Assimilation Rate

(i) Dry-Weight Basis,  $E_w$ . The data for both 14- and 28-day intervals are presented in Fig. 6 and were calculated from the original dry-weight data

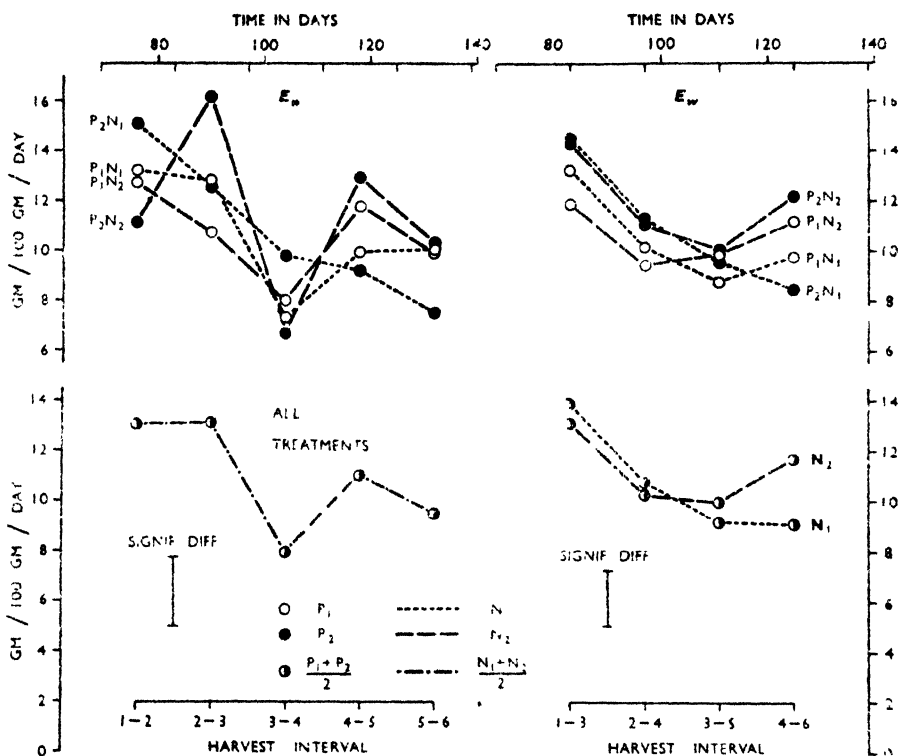


FIG. 6. Net assimilation rates ( $E_w$ ) for *Phalaris tuberosa* L. These are for 14-day (left) and 28-day intervals (right), and means appropriate to time alone and the  $N \times$  time interaction are plotted below the respective sets of harvest-class means. Significant differences (1 per cent.) are shown as vertical lines.

using equation IV. The second-order interactions were insignificant in each case: time alone was significant in the 14-day data but the  $N \times$  time interaction was significant at the 5 per cent. point in the 28-day data. The only demonstrable effect of treatment was an increase with nitrogen at harvest-interval 4-6.

The mean values of  $E_w$  for harvest-intervals 1-2 and 2-3 are each greater than those for 3-4 and 5-6. The increase from interval 3-4 to 4-5 was also significant. The time trend is somewhat similar in detail to that in the hours of bright sunshine (see Fig. 7), though the general trend is downward in the former and upward in the latter. The 28-day data indicate that the downward trend is more pronounced with low than with high nitrogen supply.

A superficial examination of the data for  $E_W$  might suggest that the other component of  $R$ , the leaf-weight ratio, did not contribute significantly to the variation in  $R$ . That this was not so is shown by the absence of a significant effect of phosphorus on  $E_W$ ; the effect of this treatment on  $R$  is primarily due to its effect on leaf-weight ratio (vide supra). Then again, for treatment  $P_2 N_1$ ,

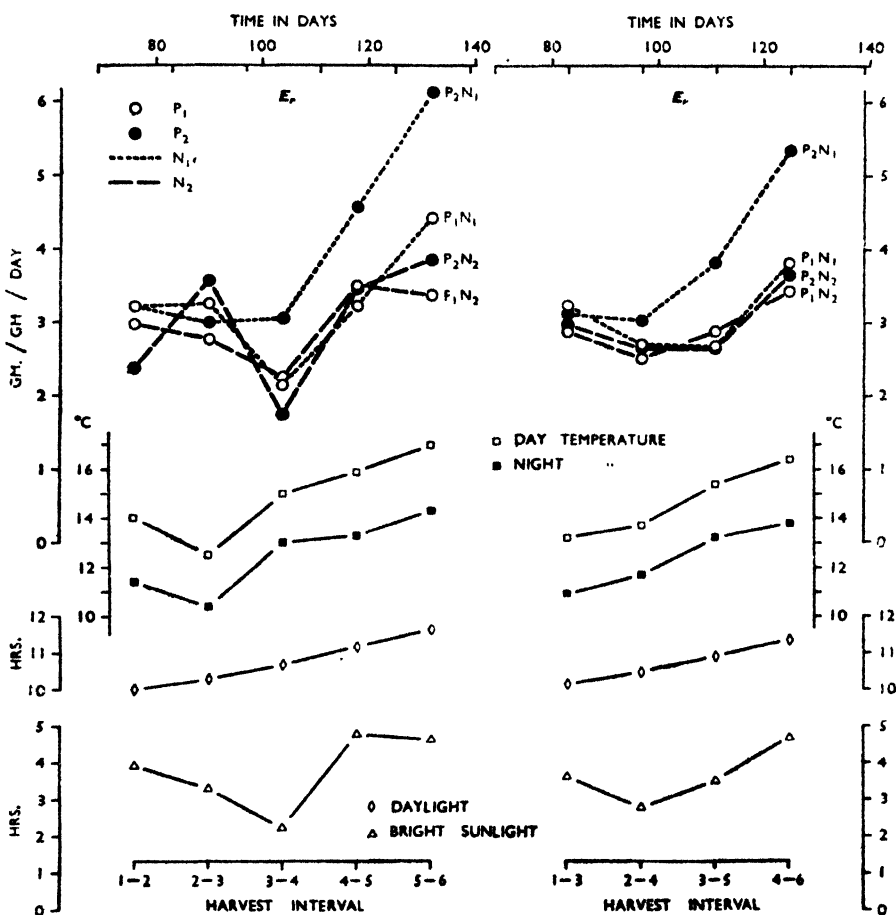


FIG. 7. Net assimilation rates ( $E_P$ ) for *Phalaris tuberosa* L. These are for 14-day (left) and 28-day intervals (right) and are plotted above mean values of available indices of temperature and light.

the over-all fall in  $R$  is 63 per cent. of its value for interval 1-2, whereas that in  $E_W$  is only 50 per cent. Other effects of time and treatment on leaf-weight ratio contribute to a lesser extent to the corresponding effects on  $R$ .

(ii) *Protein-Nitrogen Basis,  $E_P$* . These data are presented in Fig. 7 together with appropriate mean values for certain climatic indices. Values of  $E_P$  were calculated by the graphical method discussed under 'Theoretical considerations'. Although the statistical treatment of these sets of data was limited by

the absence of replication of the  $E_P$  values, certain general effects were established, and these may be checked by reference to Table VI. The general time trends in  $E_P$  are more in accord with the known effects of light and temperature on carbon assimilation than are the downward trends in  $E_W$ . It is probable, however, that the protein content of the leaves did not change greatly with time prior to harvest 1 (day 69); hence the trends in  $E_W$  and  $E_P$  would have been essentially the same for this period, and it is to be expected that both would be determined primarily by factors of the environment. The present data indicate that this relation persists for a further 70 days in the case of  $E_P$  but not with  $E_W$ .

At the higher level of phosphorus supply,  $E_P$  is depressed by increased nitrogen, and at the lower level of nitrogen the rate is increased by increased phosphorus supply. Over the period studied the values of  $E_P$  with treatment  $P_1 N_2$  were similar to, and not, as might have been expected, lower than those with  $P_1 N_1$  and  $P_2 N_2$ . Inspection of Fig. 7 shows that the above general effects of treatment are by no means consistent in time, for apart from the erratic course of the rate for treatment  $P_2 N_2$  (14-day intervals), treatment effects on  $E_P$  are negligible prior to harvest 3; thereafter, the values for treatment  $P_2 N_1$  are greater than are those for all other treatments. This fact is emphasized by the high level of significance attaching to most of the differences when these are based on the last three and the last two harvest-intervals of the 14- and 28-day data respectively (see Table VI). These effects on  $E_P$  of variation in the supply of nitrogen and of phosphorus confirm the findings of

TABLE VI  
*Effects of Treatment and Time on  $E_P$*

Differences between treatment means (14-day intervals).	Harvest-intervals 3-4, 4-5, 5-6 combined.
$P_2 N_1 - P_1 N_1 = 0.74$ } Signif. diff.	$P_2 N_1 - P_1 N_1 = 1.33^*$ } Signif. diff.
$P_2 N_1 - P_2 N_2 = 0.99^*$ } $1\% = 1.081$	$P_2 N_1 - P_2 N_2 = 1.57^\dagger$ } $1\% = 1.397$
$P_2 N_1 - P_1 N_2 = 1.02^*$ } $5\% = 0.771$	$P_2 N_1 - P_1 N_2 = 1.55^\dagger$ } $5\% = 0.996$
Differences between treatment means (28-day intervals).	Harvest-intervals 3-5 and 4-6 combined.
$P_2 N_1 - P_1 N_1 = 0.72^*$ } Signif. diff.	$P_2 N_1 - P_1 N_1 = 1.34^\dagger$ } Signif. diff.
$P_2 N_1 - P_2 N_2 = 0.84^*$ } $1\% = 0.874$	$P_2 N_1 - P_2 N_2 = 1.43^\dagger$ } $1\% = 1.236$
$P_2 N_1 - P_1 N_2 = 0.91^\dagger$ } $5\% = 0.609$	$P_2 N_1 - P_1 N_2 = 1.43^\dagger$ } $5\% = 0.861$

Harvest-interval means with appropriate significant differences.

Interval.  $E_P$

1-2	2.94	} Signif. Diff.
2-3	3.15	
3-4	2.30	
4-5	3.69	
5-6	4.45	
		$1\% = 1.209$
		$5\% = 0.862$

Interval.  $E_P$

1-3	3.05	} Signif. Diff.
2-4	2.72	
3-5	3.01	
4-6	4.07	
		$1\% = 0.874$
		$5\% = 0.609$

\* Significant at the 5 per cent. point.

† Significant at the 1 per cent. point.

Williams (1939), but it is difficult to say whether the 'internal factor' for growth is more accurately measured by leaf protein in the case of  $P_2N_1$  than it is with the other treatments. With treatment  $P_2N_1$  net protein hydrolysis was taking place in the leaves after harvest 4, and it is possible that, as in the experiments with flax and linseed, the high values of  $E_P$  with treatment  $P_2N_1$  are indicative of a reduction in the 'nitrogen status' of the experimental plants. On the other hand there is no evidence to suggest that the 'nitrogen status' was ever high with this treatment.

In spite of the general agreement between time trends in  $E_P$  and those in light and temperature, it is evident that the existence of the foregoing treatment-effects on the rate seriously limits the value of total leaf-protein as an index of the 'internal factor' for growth. Even so, it is quite clear from Fig. 7 that in this experiment the light factor was at least as important as temperature as a determinant of  $E_P$ . In most other cases temperature would seem to be the more important determinant of net assimilation rates.

## ✓ DISCUSSION

From the foregoing review and the new data presented it is apparent that net assimilation rates so far determined are subject to time trends and treatment effects which reveal the crudity of leaf area, leaf weight, and leaf protein (or nitrogen) as measures of the 'internal factor' for growth. However, the recognition of this crudity does not destroy the value of these rates, provided they are interpreted with caution.

Briggs (1928) has indicated the difficulty of distinguishing between those factors which contribute to the 'internal factor' for growth and those which make up the external complex which we call the environment. Thus, when we subject an experimental plant to artificial drought, an external factor acts via stomatal closure and a reduction in foliar hydration to limit the rate of carbon assimilation. This and all other treatments which temporarily or permanently impair the photosynthetic mechanism may be expected to depress the value of  $E$  on any basis, the depression being an approximate measure of the severity of the effect. Variation in the supply of nutrients such as nitrogen and phosphorus, on the other hand, tend to affect the amount of 'growing material' by stimulating or inhibiting meristematic activity, but should not affect the value of  $E$  provided this is based on an adequate measure of the effective 'growing material'. The depression in  $E_W$  with both deficient and excessive supplies of phosphorus during early growth stages of oats, and the absence of such effects on  $E_P$  during the same period, suggests that leaf protein is a more adequate measure of 'growing material' than is leaf weight. However, for later stages of growth even leaf protein breaks down as a basis, for  $E_P$  is then depressed both by low phosphorus and high nitrogen supplies. The evidence suggests that  $E_A$  is little affected by nitrogen supply over the ranges studied, but it may be doubted whether this remains true with more extreme nitrogen deficiency.

A further indication of the adequacy of the bases of computing  $E$  is provided by a comparison of the general time trends in  $E$  with those in climatic factors such as temperature and light intensity. Ideally, both the trend and the point-to-point fluctuations in  $E$  should be determined solely by the environment, and an obvious discrepancy between the trends will usually mean that there is an inherent trend in the estimate of  $E$ . This test is most valuable when two or more estimates of  $E$  can be calculated from a given set of data, and it is upon such tests that the generalized diagram of Fig. 8 has been constructed. This presents the time trends in  $E_A$ ,  $E_W$ , and  $E_P$  which may be expected during the growth period of an annual plant in a constant environment and with nitrogen in relatively short supply. The seedling phase A-B and the senescent phase

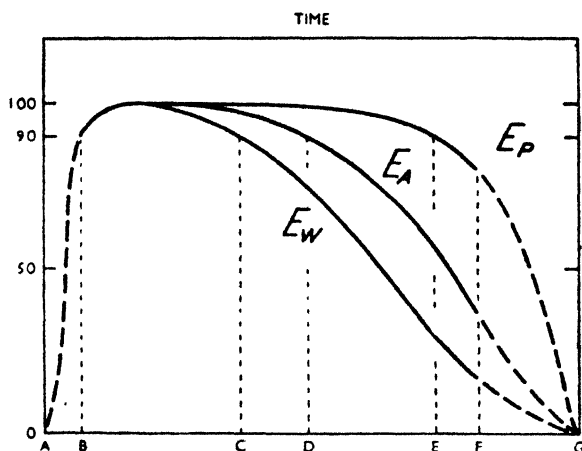


FIG. 8. Diagram showing time trends in net assimilation rates,  $E_A$ ,  $E_W$ , and  $E_P$  which may be expected in a constant environment and with nitrogen in relatively short supply.

F-G may be neglected because carbon assimilation does not then account for the bulk of the change in the dry weight of the plant. Maximum values of  $E$  are attained very soon after the seedling phase, but the period of relative constancy (here defined as a departure of less than 10 per cent. from the maximum value) varies greatly with the experimental material and with the basis of computation. In the case of  $E_W$ , this period, B-C, may be very short as in Sudan grass (Ballard and Petrie, 1936) or as long as 7 to 8 weeks in *Phalaris* (vide supra). For  $E_A$  the period of constancy, B-D, is likely to be longer than for  $E_W$ , since the dry weight per unit leaf area usually increases with time. However, there are exceptions to this, as in buckwheat (Maiwald, 1930). The data of Briggs et al. (1920a) with maize suggests that  $E_A$  was constant for at least 8 weeks, but in the majority of cases both  $E_W$  and  $E_A$  show pronounced downward trends which cannot be attributed to the environment or to the onset of senescence alone. The data from this laboratory for Sudan grass, wheat, oats, and *Phalaris* indicate that  $E_P$  is relatively constant (B-E) for almost the whole of the effective period of growth, provided only that nitrogen is in short supply. Except where a manurial treatment induces a high nitrogen

status within the plant,  $E_p$  is clearly the best available measure of  $E$ . However, it should be noted that Tiver and Williams (1943) have suggested that cytoplasmic protein rather than total leaf-protein might prove to be a more adequate basis of computation. This would involve the application of a somewhat elaborate technique (see Hanson, 1941), but it might be justified for the further analysis of the effect of nitrogen supply on  $E_p$ .

The next step in the analysis of plant growth is the determination of the relation between  $E$  and the environment. This has been attempted for  $E_A$  in maize and barley by Briggs et al. (1920a) and Gregory (1926) respectively, but these workers assumed the absence of any inherent trend in their values. Since this assumption may not have been justified, a more appropriate method would be to eliminate time trends from both dependent and independent variables (preferably by the method of regression), and then to correlate the residuals.<sup>1</sup> Even this procedure does not allow for the fact that the environment would not have the same absolute effect on  $E$  at all levels of the 'internal factor'.

Heath and Gregory (1938) rightly stress the ecological importance of the concept of net assimilation rate, but it may be doubted whether their evidence justifies the statement that 'plants as different as are barley, mangolds, cotton and tomato, and in such diverse environments as out of doors in England and in Africa and under glass in England—all have the same mean net assimilation rate during their vegetative phase'. The mean values of  $E_A$  which contribute to this *constancy* range from 0.125 to 0.720 gm./dm.<sup>2</sup>/week, and the comparison of the values is justified on the ground that  $E_A$  is independent of time during the vegetative phase of annual plants—a proposition which is as yet unproven. Heath and Gregory may only mean that values of  $E_A$  would not be very variable for different plant species grown over the same period in a given environment. However, examples which satisfy these conditions and which are included in their table show that even these differences may not be set aside with impunity. Thus Italian rye grass has a value which is 33 per cent. higher than that for cocksfoot, and the value for sugar-beet (I–III sowings) is 25 per cent. higher than that for mangolds. It is true that the superiority of sugar-beet is reduced to 5 per cent. in the case of the later sowings (IV–VI), but if any of these differences were statistically significant it would be of ecological importance because, other things being equal, its effect would be cumulative. Concerning the data presented by Heath and Gregory, little more can be said than that they are of the same order of magnitude. Much more experimental evidence will be required before it is permissible to generalize concerning the constancy or otherwise of mean net assimilation rates.

In studies of crops as they grow in the field it is impossible to obtain a quantitative recovery of the root system, hence it is important to assess the errors which may arise in growth analyses based on tops only. Thus, the exclusion of the germinating grain along with the root system may give high initial values of  $E$  because much of the dry matter of the shoot is then derived

<sup>1</sup> This has in effect been attempted by Watson and Baptiste (1938), but their data were inadequate for the purpose and gave negative results.

from the endosperm and not by direct synthesis in the shoot. Secondly, the exclusion of the root system distorts the time trends in  $E$  because the proportion of total assimilates diverted to the root system varies considerably with stage of growth.<sup>1</sup> Thus, values of  $E$  may be grossly under-estimated during early vegetative growth; they will be approximately correct for several weeks before flowering when change in weight of the root system is negligible; and they will be slightly over-estimated during the senescent phase when reserve substances are translocated from roots to shoots. Thirdly, the exclusion of the root system may obscure or exaggerate the real effects of a given treatment on  $E$  because manurial and other treatments frequently have differential effects on root and shoot growth, especially during early stages.

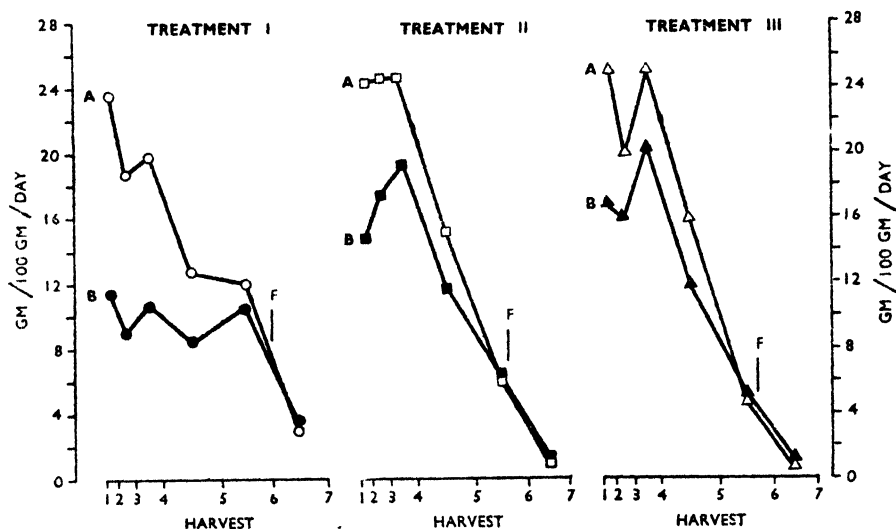


FIG. 9. Net assimilation rates,  $E_W$  for oats (Williams, 1936), the treatments being 0.008, 0.09, and 0.6 gm. P per pot. In each case curve A is based on dry weights for total plants and curve B on dry weights for shoots only.

The distortions in trend and in treatment effect are exemplified in Fig. 9, where  $E_W$  values for the whole plant are compared with those for shoots alone for the three phosphorus treatments of the second experiment with oats (Williams, 1936). In particular, the relative constancy prior to flowering of  $E_W$  (shoots only) with treatment I is seen to be spurious, as are the treatment effects at harvest interval 1-2. It must be admitted that the distortion in trend is abnormally large with low phosphorus supply because of the high values of root weight ratio with this treatment. Further values of  $E_W$  (shoots only) for three other experiments from this laboratory are presented in the supplement to this paper. Some of these exhibit the high initial values attributable to the exclusion of the germinating grain.

<sup>1</sup> The general statement which follows is based on experiments with cereals, but the principle would be valid for most annual plants.

## SUMMARY

Net assimilation rate is defined as the rate of increase in the dry weight of a plant per unit of active 'growing material', and an attempt is made to evaluate leaf area, leaf weight, and leaf protein as indices of this 'internal factor' for growth.

The derivation of the formulae for calculating mean values of relative growth rate and net assimilation rate over specified time-intervals is discussed, and the approximate nature of the formula for net assimilation rate is stressed.

A method based on graphical interpolation is described for estimating mean values of net assimilation rate in cases where it is known that considerable error may be introduced by direct use of the approximate formula. Failure to eliminate such errors may invalidate attempts to relate net assimilation rate to factors of the environment.

Following a review of the literature, a growth experiment with *Phalaris tuberosa* L. is described. The experiment was limited to the vegetative phase of growth, and nutrient treatments comprised two levels of nitrogen and two of phosphorus in all combinations. Six harvests were taken at 14-day intervals.

Tiller counts indicated that early meristematic activity was stimulated more by phosphorus than by nitrogen, and that the effect of nitrogen was delayed and was then greater at the higher level of phosphorus. These effects were reflected in the dry-weight data for total plant, leaves, and stems, but not in those for roots.

Protein nitrogen data for the leaves indicated that phosphorus is directly concerned in protein synthesis.

The data for relative growth rate and net assimilation rates (dry-weight and protein bases) are examined in detail. As in previous work from this laboratory, leaf protein proved more adequate than leaf weight as an index of the 'internal factor' for growth. This conclusion rests solely on a visual comparison of the time trends in net assimilation rates with those in temperature and hours of bright sunlight, and it would seem that very extensive data of high accuracy will be required to determine the mathematical relation of these rates to relevant factors of the environment.

In general, it may be said that leaf area and leaf weight are suitable indices of active 'growing material' only during early vegetative growth, but that leaf protein is suitable for a considerably longer period, provided that nitrogen is in short supply.

Attention is drawn to the errors in net assimilation rates based solely on sub-aerial plant parts. Such errors are inevitable in analyses of field-grown crops.

Supplementary data and some of the primary data of the *Phalaris* experiment have been deposited at the Natural History Museum, London.

The author desires to acknowledge his indebtedness to the late Dr. A. H. K. Petrie for advice during the planning of the experiment, to Professor J. G.



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## Studies on Germination and Seedling Growth

### III. Early Growth in relation to certain Aspects of Nitrogen Metabolism in the Seedling of Barley

BY

R. BROWN

*(Botany Dept., Bedford College, London)*

With seven Figures in the Text

#### INTRODUCTION

THE present investigation is intended as a contribution to the general subject of the relation between growth and metabolism during seed germination, and it is in this connexion that changes in the seedling contents of certain nitrogen fractions have been examined. During the germination of the intact seed, complex nitrogenous substances are absorbed from the endosperm, and the subsequent metabolism of these must affect the growth of the seedling profoundly. But conversely the metabolism must itself be affected by the growth and development of the system. In germination, growth occurs in a group of tissues in which the physical conditions for metabolic activity are changing, the changes being marked by an increasing water-content and by an increasing respiration rate. Thus the situation requires that the metabolism should be examined not only in relation to growth in terms of dry weight but also in relation to the water uptake and the respiration rate. Accordingly, in the present investigation, in addition to serial estimations of the levels of certain nitrogen fractions, the experimental design includes corresponding measurements of the dry weight, fresh weight, and the gaseous exchange. Moreover, the different groups of observations are repeated with two different series of cultures. For the seedling, germination in normal circumstances involves not only access to the food reserves of the endosperm, but also exposure to conditions of restricted gaseous exchange and of low water availability. The influence of these on development and metabolism may be considerable, and some independent estimate of them is clearly desirable. Therefore, in addition to the observations on seedlings from intact seeds, a second group is included based on seedlings excised immediately after the induction of germination and grown in contact with water only.

The cultures for each series are continued for 96 hours. The experiments are restricted to this interval, since they are intended to cover only the stage in development that is distinguished as germination, by which is understood the stage during which, in normal circumstances, the seedling is wholly

dependent on the food reserves of the endosperm. This stage in the conditions of the present series of experiments lasts for about 96 hours, by which time although the food reserves of the endosperm have not been exhausted there is an abundant root system and the first leaf extends beyond the coleoptile; the seedling is therefore capable of exploiting another source of nutrients.

#### EXPERIMENTAL METHODS

As indicated above, the investigation involves two cultural series, one with excised embryos and another with intact seeds. For the first, embryos were excised from seeds which had been in contact with water for 2 hours. Excisions from dry seeds were not attempted since it is difficult to remove the embryo from these without injury (Brown, 1943*a*), and throughout the investigation no observations were made on the seedlings before the beginning of germination. The technique of excision used is given elsewhere (Brown, 1943). The technique of culturing used in an earlier investigation (Brown, 1943), in which the seedlings were placed on a free water surface on specially designed floats, could not be used. The necessary materials were not at the time available and another method had to be adopted. The seedlings were placed on sintered glass discs resting on the bottom of a Petri-dish with a volume of water just sufficient to cover the lower edge of the disc. In these circumstances the seedlings were in immediate contact with water and their gaseous exchange was not seriously restricted.

At intervals of 6, 12, or 24 hours samples were taken from the cultures for the determination of one or more of the following: fresh weight, dry weight, the gaseous exchange, and the contents of certain nitrogen fractions. When the sample was taken from a culture of intact seeds, since data regarding the seedling alone were required, the seedling was separated from the endosperm by the standard excision technique. Fresh weight was determined immediately after sampling; the dry weight after heating for 24 hours at 80° C., and the gaseous exchange in a Barcroft respirometer, the details in each case being the same as those given for an earlier investigation (Brown, 1943).

The analyses involved serial estimations of total, soluble, amino, amide, and ammonia nitrogen, the techniques being based on those used by Richards and Templeman (1936) in the determination of the same fractions in the leaf of barley. Total nitrogen was determined on aliquots of a suspension of embryo tissue that had been ground to a fine paste in a mortar; total soluble, amide, and ammonia nitrogen were determined in a clear filtrate of the same suspension after precipitation of the proteins with trichloroacetic acid. Total and soluble nitrogen in the suspension and filtrate respectively were estimated with the micro-Kjeldahl technique, using the reduced-iron modification of Pucher, Leavenworth, and Vickery (1930) on a micro scale; ammonia nitrogen by treatment of the filtrate with magnesia cream, removing the ammonia in a current of air in the cold and collecting it in standard acid according to the

method of Wolff (1928); amide nitrogen by hydrolysing in 10 per cent. sulphuric acid for 4 hours, neutralizing with saturated sodium hydroxide solution, and then proceeding as with the estimation of ammonia. Amino nitrogen was determined by the adaptation of the formol titration technique of J. H. Brown (1923). A weakly alkaline suspension of ground seedling tissue was treated with barium chloride and filtered. After neutralization of the filtrate to pH 8, excess of formaldehyde at the same pH was added, and the whole then titrated back with standard sodium hydroxide solution to the original acidity.

All the observations with each cultural series referring to seedlings of the same age were not of course made with the same sample, nor was the size of the sample constant. The sample size in each connexion is indicated below by the figures in brackets. A different sample was used for each of the following pairs of determinations; fresh and dry weight (50); total and soluble nitrogen (50); amide and ammonia nitrogen (100); and a separate sample again for each of the following, the rate of oxygen uptake (25), the rate of carbon dioxide production (25), and the content of amino nitrogen (200).

The cultures were maintained in the dark at 22° C.

In this paper the term 'isolated seedling' refers to the material that is excised at 2 hours, and subsequently cultured with water only, 'attached seedling' to that derived from the culture of intact seeds and separation from the endosperm effected at the time of sampling. In certain connexions observations on the attached group of seedlings are made after a 4-hour period following excision, and this material is referred to as 'detached seedlings'.

Each of the protein and total nitrogen and of the dry and fresh weight values given in the next section are the means of two estimations, the rest represent single observations.

### EXPERIMENTAL RESULTS

Below the results of observations on growth, on the gaseous exchange, and on the seedling contents of certain nitrogen fractions are given separately, the corresponding data for attached and isolated seedlings being presented together. In each isolated seedling experiment the first observation is made 10 hours after excision is effected; and the changes that occur during this interval are of course given by a comparison of the first isolated with the first attached seedling observation made 2 hours after the parent culture is established. In order to facilitate the comparison the first attached seedling result is reproduced in the tables as the first isolated seedling observation for material 2 hours old. The first actual determination after the establishment of the isolated seedling culture appears as the second entry in the tables.

*Fresh and dry weight at successive stages of development.* The fresh and dry weights of isolated and attached seedlings sampled at intervals of 12 hours over a period of 96 hours are shown in Table I.

TABLE I

*Fresh weight (FW) and dry weight (DW) (mg. per seedling) of attached and isolated Seedlings at successive Stages of Development*

Age of seedling (hours).	Isolated.		Attached.	
	FW.	DW.	FW.	DW.
2	2.76	1.6	2.76	1.6
12	5.2	1.23	3.71	1.57
24	8.52	1.26	4.25	1.52
36	7.1	1.2	5.61	1.7
48	11.34	1.24	6.4	1.77
60	9.37	1.13	11.84	2.14
72	9.34	1.14	18.36	3.12
84	—	—	29.92	4.25
96	10.64	1.18	40.3	5.88

The differences between the two cultural series are considerable. Dry weight in the attached seedling decreases during the first 24 hours, increases slowly during the next 24 hours and rapidly during the last 48 hours; in the isolated seedling, on the other hand, it decreases sharply during the first 10 hours, and slowly but continuously thereafter. Fresh weight in the attached seedling increases slowly during the first 48 hours and rapidly during the last 48 hours; in the isolated seedling it increases rapidly during the first 48 hours and thereafter remains more or less constant.

It is evident from Table I that both fresh and dry weight increase in the attached seedling occurs in two phases, and this feature is well shown by Fig. 1 in which the logarithmic values are plotted against time. This figure, however, also suggests that the increase within each phase is exponential—the line of closest fit calculated from a linear regression equation fits the experimental points in certain cases remarkably closely. The equations have been calculated from the first 5 and from the last 4 values in each series (thus the observation made at 48 hours is included with the first and not with the second group). The equations together with the relevant correlation coefficients are:

For fresh weight 2–48 hrs.  $\log_{10} W = 0.6388 + 0.00788 (X - \bar{x})$ ,  $r = 0.987$  (with 3 *df* significant at 1 per cent. level).

For fresh weight 60–96 hrs.  $\log_{10} W = 1.3544 + 0.01508 (X - \bar{x})$ ,  $r = 0.999$  (with 2 *df* significant at 1 per cent. level).

For dry weight 2–48 hrs.  $\log_{10} W = 0.2106 + 0.00100 (X - \bar{x})$ ,  $r = 0.721$  (with 3 *df* not significant at 5 per cent. level).

For dry weight 60–96 hrs.  $\log_{10} W = 0.5556 + 0.01209 (X - \bar{x})$ ,  $r = 0.993$  (with 2 *df* significant at 5 per cent. level).

An acceleration in the rate of dry weight increase after germination for 2 days at 25° C. has been reported by Barnell (1937). This is confirmed by the present series of data, but whereas dry weight increases exponentially in the second phase, there is little evidence that it does so in the first. On the other hand, fresh weight increase is undoubtedly exponential in both phases. The acceleration that occurs after 48 hours affects fresh and dry weight differentially, with fresh weight it doubles the rate of increase, and with dry weight it

increases it at least tenfold. The relative rate of fresh weight increase, however, always exceeds the corresponding rate of dry weight increase.

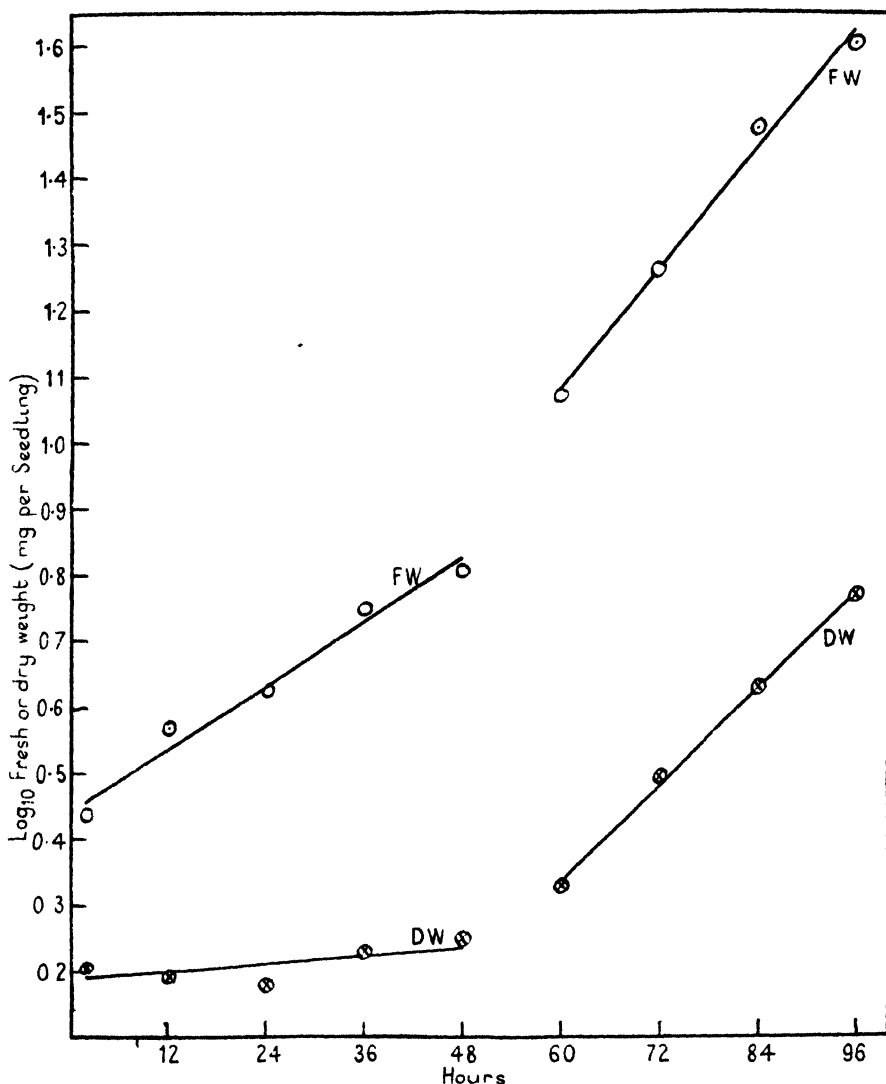


FIG. 1.  $\text{Log}_{10}$  of fresh weight (FW) and dry weight (DW) of attached seedlings at successive stages of germination. The curves connect values calculated from the regression equations given on page 76.

*Changes in the gaseous exchange with time.* The gaseous exchange of the isolated seedling may be determined directly, but that of the attached seedling can only be estimated indirectly from the whole seed exchange. The whole seed data must be adjusted to provide for the respiration of the endosperm and for the effect that attachment to the rest of the seed is likely to

have on the seedling exchange. The necessary numerical values may be obtained by determining the exchange of the whole seed, of the excised endosperm, and of the detached seedling. The results of an experiment designed to provide such data for successive stages of germination are given in Table II.

TABLE II

*Gaseous Exchange (mml. per hr.) of intact Seed, of detached Seedling and of excised Endosperm, the Separation of the two Parts of the Seed being made at successive Stages of Germination*

Age of culture (hours).	Intact seed.		Seedling.		Endosperm.	
	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
2	2.16	0.8	2.54	2.41	0.45	0.43
24	3.43	1.7	3.99	3.68	2.91	2.69
36	5.3	4.7	7.8	8.23	3.88	4.88
48	10.2	11.0	12.3	13.4	4.94	5.17
60	15.9	16.7	13.17	14.5	4.75	5.12
72	25.6	24.6	26.7	26.0	4.77	5.54
96	39.0	40.8	40.42	41.2	6.14	5.92

The endosperm values given in Table II, since they were determined incidentally to the main purpose of the investigation, are not considered further (although they are of some significance in relation to comparable data obtained by Stoward (1907), Merry and Goddard (1942), and Taylor (1941)). The intact seed values, however, require some comment since they differ from comparable data obtained by James and James (1940). The results of the present series indicate little change in the rate of oxygen uptake between 2 and 24 hours, an increase between 24 and 36 hours, and a further increase between 36 and 48 hours. They do not suggest, what the data of James and James indicate, that the rate increases with constant acceleration up to 48 hours. The different results are undoubtedly due to different experimental conditions; in these experiments the seeds were in contact with water, but in those of James and James, since they were pressed into moist sand, they probably were not, and the seed coats probably had a lower water-content with a higher permeability to gases. Thus, the difference is probably due to different conditions with respect to the diffusion of gases to the seedling. The different values of the respiratory quotient in the first 24 hours of germination are consistent with this interpretation (Brown, 1943).

Table II shows that the combined values of seedling and endosperm always exceed those for the intact seed. Clearly separation of the two parts enhances the exchange of each. Since the respiratory characters of both components are similar, it may be supposed that the increase is relatively the same with each; in which case, the attached seedling exchange is given by reducing the detached seedling values by an amount proportional to the difference between the intact seed and the combined seedling and endosperm values. In Table III attached seedling values calculated in this way are compared with directly determined isolated seedling values.



TABLE III  
Gaseous Exchange (mml./seedling/hr.) of Attached and Isolated Embryos at successive Stages of Germination

Age of seedling (hours).	Isolated.		Attached.	
	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>
2	—	—	1.8	0.68
12	3.2	3.7	—	—
24	3.7	4.6	2.0	0.86
36	3.4	4.9	3.5	2.8
48	4.8	6.0	7.3	7.9
60	3.7	4.8	12.9	11.7
72	3.7	5.0	20.3	21.7
84	2.6	4.1	—	—
96	3.2	4.6	35.7	40.5

The rates of oxygen absorption of Table III and the same data for the detached seedling of Table II, together with the corresponding respiratory quotients, are presented graphically in Fig. 2. The reason for considering the detached seedling values in this connexion is indicated in the next section. The differences between the two cultural series are considerable. The rate of oxygen uptake of the isolated seedling increases slowly but continuously from 2 to 48 hours and then decreases; but that of the attached seedling, while it increases slowly during the first 24 hours, increases rapidly and continuously during the rest of the experimental period. The differences between the corresponding attached and isolated seedling values vary according to the period of development. During the first 36 hours the rate of absorption of the isolated seedling is greater than that of the attached, but thereafter the difference is in favour of the attached and becomes increasingly greater with time. The respiratory quotient of the attached seedling is high during the first 24 hours, drops to about 1.0 in the next 12 hours, and maintains approximately the same value until 96 hours; the quotient of the isolated seedling, on the other hand, which at the time of excision is 1.0, falls gradually throughout the experiment to a little more than 0.6. It may be noted here that at all stages of development the rate of oxygen uptake of the detached seedling is higher than that of the attached; and whereas the respiratory quotient of the latter is at first 2.3, that of the former remains throughout at about 1.0.

*Change in the seedling contents of various nitrogen fractions with time.* As indicated in a previous section in the determination of the total and soluble nitrogen fractions, the reduced-iron modification of the Kjeldahl technique was adopted to provide for the possible occurrence of nitrates. It was found, however, that this provision was unnecessary. After the amide estimation the ammonia-free alkaline solution was always treated with Devarda alloy and always with the same result, that no further ammonia could be detected. Routine estimations for free ammonia were also made on each sample. The results from these were not entirely negative, but they were not well defined. When free ammonia was apparently present the amount was too small for accurate estimation with the method used. Moreover, with the isolated

seedlings there was always some slight discoloration over the surface of the scutellum, induced no doubt by bacterial contamination, and the slight traces of ammonia observed with this series might have been due to this. Certainly in no instance were large quantities found. Accordingly no significance is

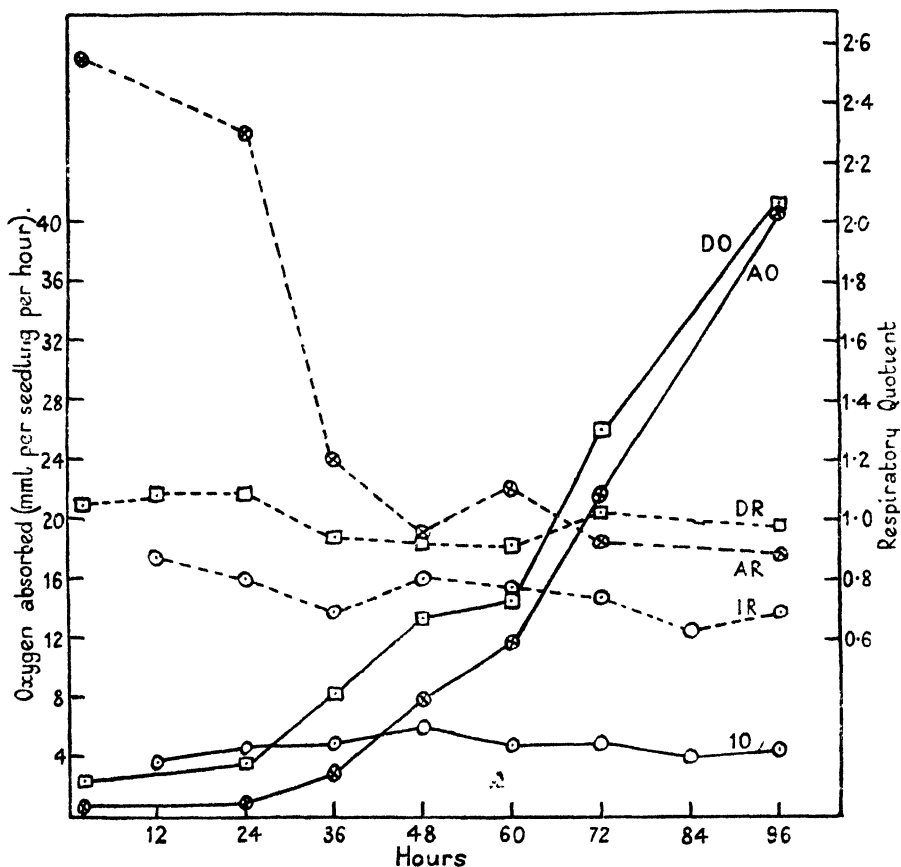


FIG. 2. Rates of oxygen uptake (O) and respiratory quotients (R) of attached (A) of detached (D) and of isolated seedlings (I) at successive stages of germination.

attached to these doubtful results and they are not presented along with the other data.

The data given below are based on estimations of total, soluble, total amino, and amide nitrogen fractions. From these other fractions have been derived; protein nitrogen by deducting soluble from total nitrogen; non-amide amino nitrogen by deducting amide nitrogen from total nitrogen; and residual soluble nitrogen by deducting the sum of non-amide amino nitrogen and double the amide nitrogen from total soluble nitrogen.

The data are presented in two groups, those referring to the total soluble and protein fractions are given in Table IV, and those referring to the constituents of the soluble fraction in Table V.

TABLE IV

*Total, Soluble, and Protein Nitrogen (mg. per Seedling) in Isolated and Attached Seedlings at Successive Stages of Development*

Age of seedling (hours).	Isolated.			Attached.		
	Total N.	Sol. N.	Prot. N.	Total N.	Sol. N.	Prot. N.
2	0.08	0.016	0.064	0.08	0.016	0.064
4	—	—	—	0.08	0.016	0.064
6	—	—	—	0.078	—	—
8	—	—	—	0.083	0.016	0.067
10	—	—	—	0.085	—	—
12	0.076	0.011	0.065	0.084	0.017	0.067
18	—	—	—	0.087	0.019	0.068
24	0.076	0.013	0.063	0.088	0.019	0.069
30	—	—	—	0.086	0.018	0.068
36	0.077	0.013	0.064	0.085	0.02	0.065
42	—	—	—	0.091	0.023	0.068
48	0.08	0.022	0.058	0.096	0.021	0.075
54	—	—	—	0.096	0.022	0.074
60	0.073	0.024	0.049	0.126	0.045	0.082
66	—	—	—	0.132	0.047	0.085
72	—	—	—	0.169	0.054	0.115
78	—	—	—	0.189	0.056	0.133
84	0.077	0.028	0.049	0.192	0.054	0.138
90	—	—	—	—	—	—
96	0.073	0.026	0.047	0.272	0.078	0.194

The effect of attachment on the seedling content of the fractions of Table IV is pronounced. The total nitrogen of the attached seedlings starts increasing after about 8 hours of germination, continues to increase slowly during the following 48 hours, and rises rapidly during about the final 48 hours; that of the isolated seedling on the contrary drops sharply during the first 10 hours and then probably remains more or less constant. Soluble nitrogen in the attached seedling follows a similar course to total nitrogen, but in the isolated seedling it does not; it drops during the first 12 hours, remains more or less constant during the following 24 hours, rises sharply during the next following period of 24 hours, and then continues to increase slowly. Protein nitrogen in the attached seedling follows a similar course to the other two fractions, but in the isolated seedling again the cycle of changes differs from those of the other two; there is little or no change for about 36 hours, there is then a sharp decrease which occupies the next 24-hour period, and then possibly a period of slow decrease.

In Fig. 3 the logarithmic values for the protein and total nitrogen data ( $\times 100$ ) of the attached seedling are plotted against time. The increase in two distinct phases indicated by the Table IV is emphasized by the curves of Fig. 3. Moreover, the linear relation to which the logarithmic values conform suggest an exponential form of increase. Each set of data have been divided into two groups, the first including the values for 54 hours, and the second beginning with the value for 60 hours; regression equations have been

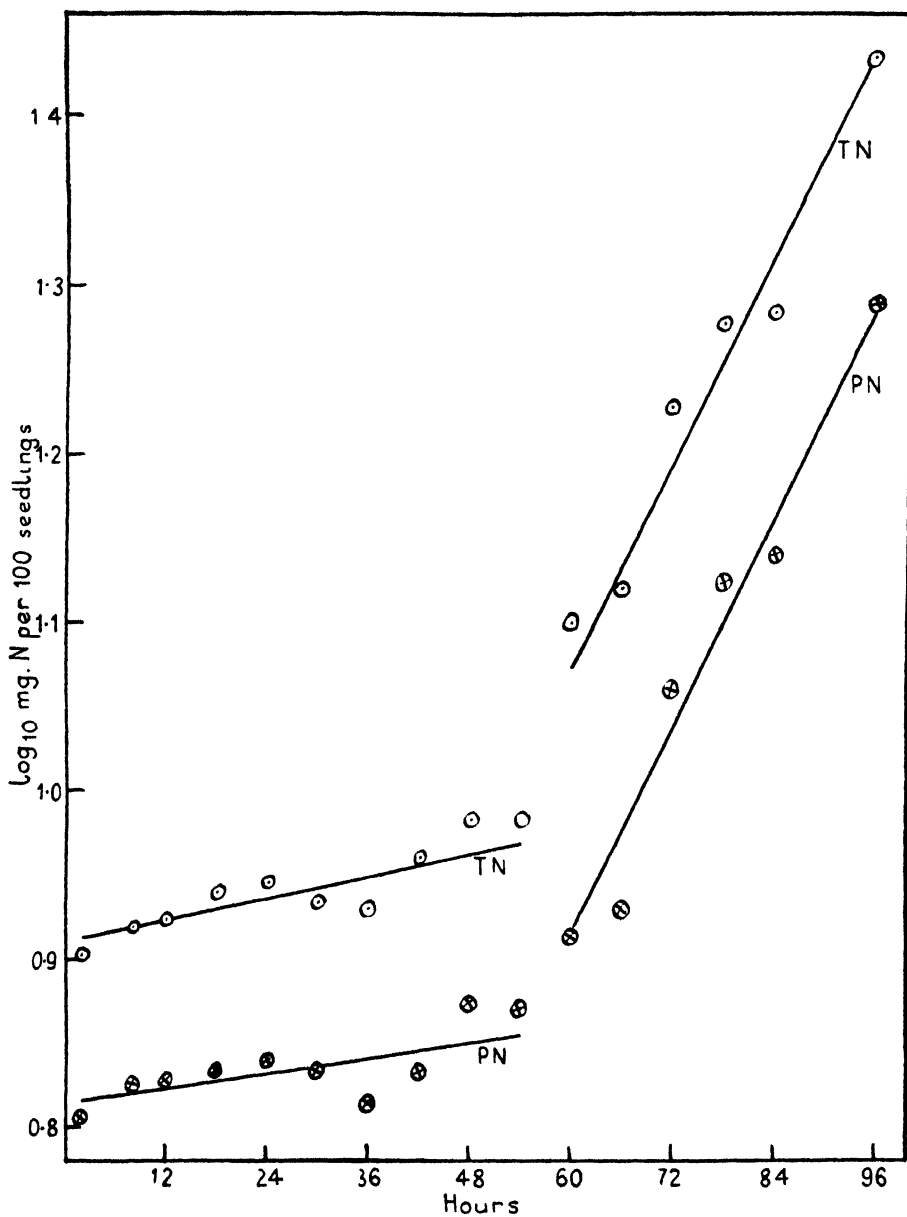


FIG. 3.  $\text{Log}_{10}$  of protein (PN) and total nitrogen (TN) of attached seedlings at successive stages of germination. The curves connect values calculated from regression equations given on page 83.

calculated for each group, and together with the appropriate correlation coefficients they are:

For total N. 2–54 hrs.  $\log_{10}N = 0.9367 + 0.001040 (X - \bar{x})$ ,  $r = 0.880$  (with 7 *df* significant at 5 per cent. level).

For total N. 60–96 hrs.  $\log_{10}N = 1.2037 + 0.01008 (X - \bar{x})$ ,  $r = 0.986$  (with 5 *df* significant at 1 per cent. level).

For protein N. 2–54 hrs.  $\log_{10}N = 0.8314 + 0.000775 (X - \bar{x})$ ,  $r = 0.633$  (with 7 *df* not significant at 5 per cent. level).

For protein N. 60–96 hrs.  $\log_{10}N = 1.0464 + 0.01037 (X - \bar{x})$ ,  $r = 0.990$  (with 5 *df* significant at 1 per cent. level).

Except for protein in the first phase, increase within the other three groups is probably exponential. The acceleration which occurs between 54 and 60 hours enhances the relative rate of increase for total N about 10 times and for protein N about 15 times. Further, while it is possible that during the first phase the relative rate of increase for total N is greater than that for protein N, during the second phase the rates are clearly more or less the same (statistically the difference between them is not significant).

TABLE V

*Total Amino N (A), Non-Amide Amino N (NA), Amide Amino N (AA), Residual Soluble N (RSN), in isolated and attached seedlings (mg. per seedling) at Successive Stages of Development*

Age of seedling (hours).	Attached.				Isolated.			
	A	AA	NA	RSN	A	AA	NA	RSN
2	0.002	0.0018	0.0002	0.012	0.002	0.0018	0.0002	0.012
12	0.0039	0.0019	0.002	0.011	0.00236	0.00081	0.00149	0.0038
24	0.0055	0.0012	0.0043	0.0123	0.00276	0.00131	0.00145	0.0089
36	0.0073	0.0014	0.0059	0.0113	0.0042	0.00193	0.00227	0.00687
48	0.011	0.0046	0.0064	0.0054	0.00547	0.00227	0.0032	0.0142
60	0.015	0.0059	0.0091	0.0241	0.00667	—	—	—
72	0.016	0.0074	0.0086	0.0297	0.00557	0.00379	0.00178	—
84	0.0184	—	—	—	0.00749	0.00514	0.00235	0.01537
96	0.0228	0.0093	0.0135	0.0459	0.00828	0.00683	0.00145	0.01092

The attached and the isolated seedling data of Table V are presented graphically in Figs. 4 and 5 respectively. A comparison between the seedling contents of all the soluble fractions of Table V of the two series shows that this is always highest in the attached seedling, and also that the course of change is different in the two series except with respect to total amino nitrogen. With this fraction the content in both series increases uniformly throughout the developmental period. Residual soluble N in the attached seedling increases in two phases, in the isolated it decreases during the first 10 hours, remains more or less constant during the following 24 hours, increases during the next 12 hours, and remains apparently more or less constant during the final 48 hours. Non-amide amino nitrogen in the attached seedling follows a course similar to that of total amino nitrogen, but in the isolated it increases

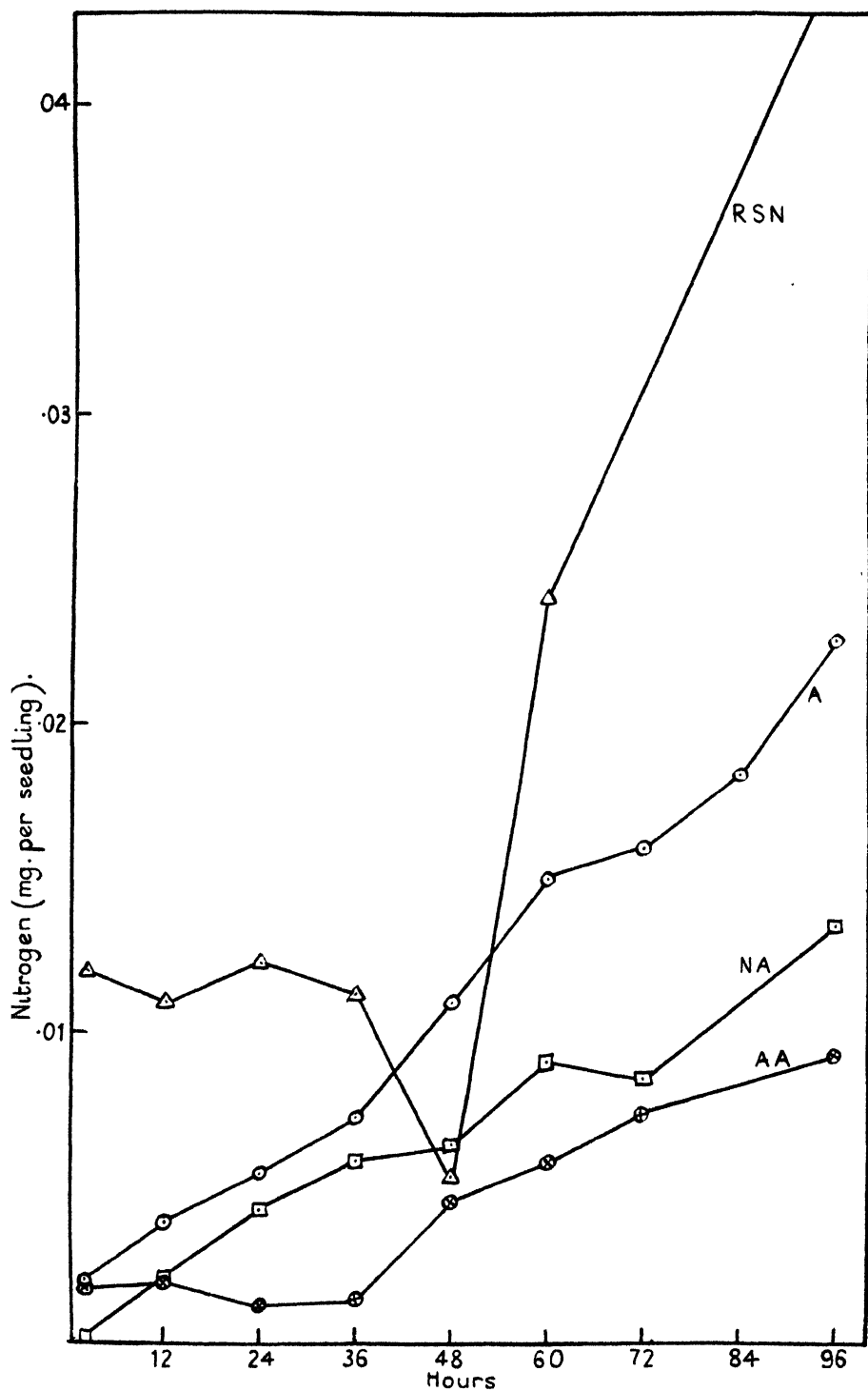


FIG. 4. Attached seedling: content of total amino (A), amide amino (AA), non-amide amino (NA), and of residual soluble (RSN) nitrogen fractions at successive stages of germination.

for 2 to 48 hours and thereafter tends to decrease. The amide fraction in the attached seedling drops between 2 and 24 hours, increases slightly between 24 and 36 hours, and thereafter increases uniformly and rapidly; in the isolated seedling it increases uniformly throughout the whole experimental period.

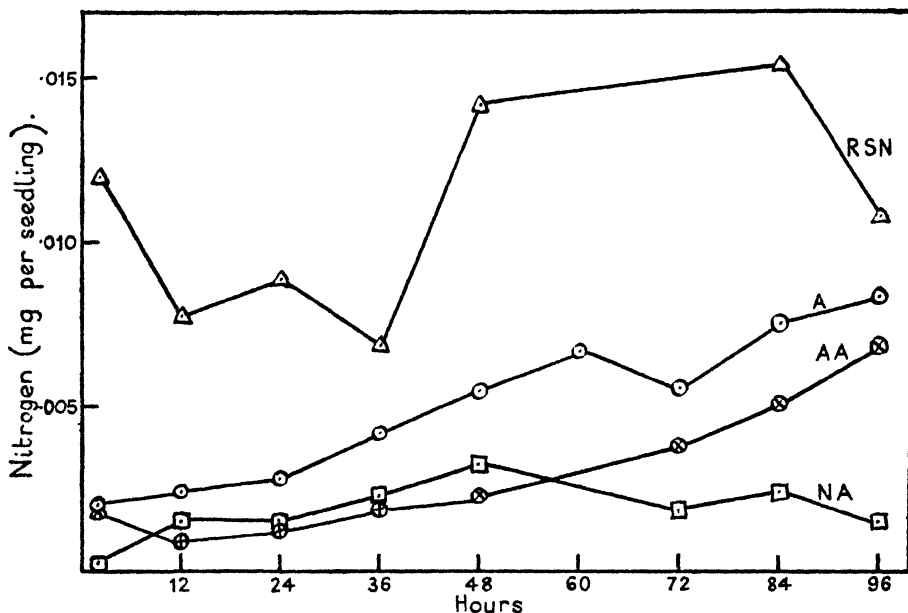


FIG. 5. Isolated seedling—content of total amino (A) amide amino (AA) non-amide amino (NA) and of residual soluble nitrogen (RSN) fractions at successive stages of germination.

#### DISCUSSION OF RESULTS

The development of both series involves progressive cell extension. This is indicated by the data of Fig. 6, which show that with isolated seedlings the water-content per unit dry weight increases over a period of 48 hours, and with attached seedlings over 96 hours. The full imbibitional pressure of a stable colloidal system might be expected to be satisfied much more rapidly than this, and evidence has been presented elsewhere to show that the limiting water-content of the colloidal system as such is in fact established within 2 hours of exposure to free water. It is evident, however, from the data of Table I that the process of cell extension does not involve simply the absorption of water into a stable system. With attached seedlings between 2 and 48 hours there is a fourfold increase in the absolute water-content, but throughout this period, since the fresh weight is increasing exponentially, the rate of water absorption is not decreasing with time. Thus the position indicates that in spite of the continued absorption of water, the suction-pressure does not decrease. Clearly such a situation implies that cell extension is accompanied by metabolic changes, the rates of which control at least partially the rate of water-absorption.

Fig. 6 shows that the water-content in terms of percentage dry weight of the isolated seedling is always greater than that of the attached, and Table I that the fresh weight of the isolated seedling is the greater until 48 hours, but that thereafter the difference is in favour of the attached. Evidently the water-absorption of the isolated is the more rapid until 48 hours; but after this stage water-absorption with cell extension increases in the attached seedling, whereas it more or less ceases in the isolated seedling. Since the comparative

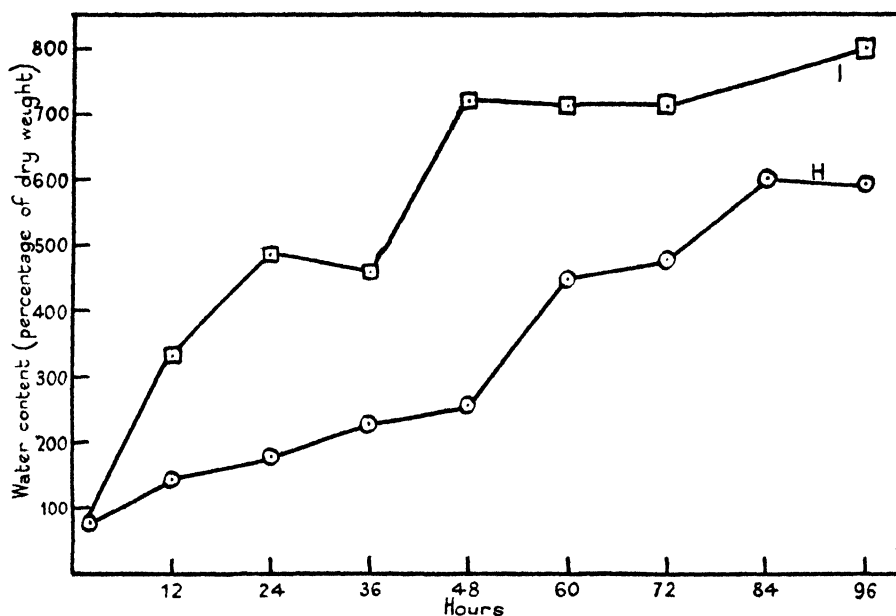


FIG. 6. Water-content of isolated (I) and attached (A) seedlings at successive stages of germination.

position changes markedly after 48 hours, the factors that determine the differences in water-absorption and cell extension may be considered for the two cultural phases separately.

In the first phase, since cell extension involves metabolic changes in the system, water-absorption is no doubt determined by internal as well as external factors, and the differences in the rate of water-absorption may therefore be considered in relation to (1) nutrient supply, (2) gaseous exchange, (3) the action of inhibitors, and (4) the level of water availability.

The data of Table I show that with the attached seedling, where the absorption of water is slow, some nutrients are being transferred to the seedling from the endosperm. The effect of the accumulation of these in the seedling is in any event not likely to depress water-absorption, and one piece of experimental evidence is available which shows that in fact it does not. In an earlier paper of this series (Brown, 1943), measurements are given of the growth of isolated seedlings cultured on a nutrient medium containing sucrose, and they indicate that in these circumstances the water-content tends to



increase rapidly during the first 48 hours, and thereafter relatively more slowly; the effect is thus the same as with no nutrient.

It is possible that cell extension is in some way dependent on respiration, in which case any factor which affected the respiration rate might also affect the rate of water-absorption. Elsewhere (Brown, 1943) it has been shown that excision raises the respiration rate (Fig. 2) by removing a barrier—the seed-coat membranes—to the free diffusion of gases between the seedling and the atmosphere. But the data for the attached seedling suggest that an enhanced respiration does not promote a corresponding increase in cell extension and therefore water-absorption. With the attached seedling (Fig. 2) between 2 and 24 hours the quotient is high and the rate of oxygen uptake low, the former indicating that the latter is due to a restricted exchange imposed by intact seed-coats (Brown, 1943). Between 24 and 36 hours the quotient falls and the rate rises, suggesting that in the interval the membranes are broken, an inference which is confirmed by direct observation since it is at this time that the extension of the root begins. Fig. 1, however, shows that between 24 and 36 hours the rates of water-absorption and respiration do not increase together, the conclusion being that in the attached seedling, and therefore probably also in the isolated one, water-absorption and cell extension are not limited by the rate of respiration.

The relatively low absorption of the attached seedling may be due to an inhibitor, but if so, the rapid absorption of the attached seedling must be due to the removal of it. Certain evidence discussed below shows that considerable leaching is induced from seedling tissues when these are exposed to water immediately after excision at an early stage of development. An effect of excising seedlings at 2 hours and transferring them to a free water surface might therefore be to remove an inhibitor along with other solutes. There is no evidence to show that such an effect is not in fact involved and it may be a contributory factor. On the other hand, there is strong evidence to show that most of the difference between the two series is due to another variable. The seed-coat membranes are not freely permeable to water and the supply to the attached is consequently considerably lower than it is to the isolated seedling. At 2 hours both seedling series are in the same developmental state, and the immediate effect of raising the level of availability must therefore be to promote an enhanced water uptake. Moreover, the data of Table VI, which show the effect on fresh weight of exposing the seedling to water for 4 hours immediately after excision at successive stages of germination, indicate that the influence of the seed-coat membranes on absorption persists throughout the development of the seedling.

At all stages the effect of transferring the seedling to water is to increase the fresh weight. Thus the relatively slow rate of water uptake of the attached seedling in the first phase may be attributed to a low level of water availability. On the other hand, although the rapid uptake of the isolated seedling is undoubtedly related to the effect of separation from the seed-coats, it may be noted that pronounced acceleration in water uptake (Fig. 1) with the attached

seedling does not occur until 48 hours, which is at least 24 hours after the seed-coat is broken in normal germination. Evidently the extension of the root only leads to the exposure of a limited area of the seedling, over which absorption is not sufficiently rapid to satisfy the full suction pressure of the tissues. A comparison between the gaseous exchange rates of attached and detached seedlings in Fig. 2 supports this conclusion. Even after 24 hours, when a more rapid oxygen uptake coincides with the breaking of the seed-coat, the complete separation of the seedling from the rest of the seed leads to a still further increase in the rate of uptake.

TABLE VI

*Fresh Weight (mg. per seedling) at successive Stages of Germination immediately after Separation from the Endosperm (A), and again after Exposure to Water for 4 Hours (D)*

Age of seedlings (hours).	A	D
2	2.76	3.85
36	5.61	7.25
60	11.84	16.50
72	18.36	31.90
96	40.30	59.03

In the attached seedling the large increase in fresh weight that occurs after 48 hours, together with the increase in water-content that occurs during this final period, suggests that cell extension proceeds during this phase more rapidly than it does during the first. The origin of this accelerated development cannot be determined from the present series of data. In the isolated seedling after 48 hours water-content remains constant, and there is no further change in fresh weight. Below it is shown that cell extension involves the formation of additional non-labile cell components, and the cessation of growth after 48 hours may therefore be attributed partly to the fact that nutrients are not supplied to the seedling, although other conditions, such as separation from a source of accessory growth factors are no doubt also involved. The extension before 48 hours occurs no doubt at the expense of reserves already present in the dormant seedling.

In the course of development there are similar changes in both series in dry weight and total nitrogen. With isolated seedlings both decrease sharply during the first 12 hours, and dry weight decreases slowly during the final 84 hours, while total N remains more or less constant; with attached seedlings both increase slowly during the first 48 hours and rapidly thereafter. With the isolated seedling the first phase of rapid decrease is undoubtedly due to leaching, since the quantity of total nitrogen lost is entirely accounted for by a corresponding decrease in the quantity of soluble nitrogen (Table IV), and two of the component groups of this fraction also decrease during the same interval (Fig. 5). The position with regard to total nitrogen indicates the same cause for the decrease in total dry weight, although other than nitrogenous components must also be involved since the nitrogen loss corresponds to a

small proportion of the decrease in dry weight. The slow decrease in dry weight of the final 84 hours must be attributed to other factors, since, as shown by the data of Fig. 5, the contents of soluble nitrogenous materials increases during this time. Moreover, as indicated by the data of Table VII, leaching losses only occur when the seedling is excised within the first 36 hours of germination.

TABLE VII

*Dry Weights (mg. per seedling) at different Stages of Germination, immediately after Separation from the Endosperm (A), and again after Exposure to Water for 4 Hours (D)*

Age of seedlings (hours).	A	D
2	1.60	1.36
36	1.70	1.58
60	2.14	2.17
72	3.12	3.75
96	5.88	5.90

Evidently, during development, there is a change in the state of the protoplast system which secures the retention of solutes against a concentration gradient. Respiration continues vigorously throughout the experimental period (Fig. 2), and this must contribute to a reduction in dry weight when the absorption of nutrients cannot occur.

In the attached seedling the increase in dry weight in the first phase only sets in after a preliminary period of slight decrease lasting for about 24 hours (Table I). At the same time total nitrogen does not decrease, and the fall in dry weight is therefore probably not due to a diffusion of solutes into the endosperm but to a dissipation of metabolites in respiration at a time when absorption is slow. In spite of this early difference, however, the trend with both total nitrogen and dry weight is the same, and the changes in the rates of increase imply corresponding and parallel changes in the rates of absorption. Since nitrogen increase begins within a few hours (probably 6) after the exposure of the whole seed to water, it is probable that the absorption of other substances begins at the same time. The results of Avery, Creighton, and Shalucha (1940) with maize are consistent with this conclusion, since they indicate that the absorption of auxin also begins about 10 hours after the induction of germination. After solute absorption begins it evidently proceeds at a slow rate until the rapid phase sets in after 48 hours. This result is in agreement with an earlier one of James (1940), who from an examination of the changes in the contents of various carbohydrates concluded that the absorption of sucrose from the endosperm is accelerated after a period of slow absorption lasting for about 2 days. The rate of nutrient absorption in the seedling evidently follows fairly closely that of water-absorption, and a consideration of the nature of the nutrient supply suggests that, as with water so with nutrients, the rate of absorption is not determined simply by the rate of supply. The endosperm consists mostly of inert tissues, and the mobiliza-

tion of the food reserves that it contains depends primarily on the supply of hydrolytic enzymes from the seedling and the aleurone layer. Clearly it is highly improbable that the supply of soluble nutrients from such a system can increase with constant acceleration, or that it can be subject to an abrupt stimulation effective within a period of 6 hours. Total nitrogen increases exponentially in both phases, and the transition from the first to the second phase is complete within 6 hours (Fig. 3), and it would therefore seem that the absorption of nitrogenous substances is determined largely by the growth reactions of the seedling. Total dry weight increase does not necessarily indicate a corresponding absorption, since some of the nutrients absorbed must necessarily be dispersed in respiration. Nevertheless, the parallel between dry weight and total nitrogen increase suggests that all nutrient absorption is largely determined by changes in the developmental state of the seedling. Such does not of course imply the position that the amount absorbed is independent of the volume of supply. At all stages the quantity accruing to the seedling must be a resultant of the resistance of the seedling tissues to solute diffusion and of the difference in solute concentration between seedling and endosperm.

Protein changes with total nitrogen and dry weight only in the attached seedling. In the isolated seedling protein nitrogen does not change until about 36 hours, when rapid hydrolysis leads to a reduction in protein and a corresponding increase in soluble nitrogen. The period of rapid hydrolysis only lasts, however, for about 24 hours, after which if it occurs at all it continues at a very much slower rate. In the attached seedling protein nitrogen increases throughout the experimental period. At no stage is there any indication of hydrolysis, and protein formation apparently begins as soon as nitrogen absorption does at about 8 hours (Table I); after this, the phases of increase correspond exactly with those of total nitrogen and dry weight. Further, during the second phase both protein nitrogen and dry weight increase exponentially, each with a constant relative rate of increase. This is of some significance in relation to the nature of the growth process with which the increases are associated.

Above it is shown that during the second phase cell extension proceeds more rapidly than it does in the first, and it is during this phase that the extension of the coleoptile and the first leaf is most prominent. Throughout the whole experimental period there is little or no indication of cell division, either in the extending organs or at the meristematic apex of the stem. Some division does apparently occur at the apex of the root, but the additional tissue formed by cell division must be an extremely small proportion of the whole, since the ratio of root weight to the weight of the rest of the seedling is only about 1 : 10. Thus the accumulation of dry matter and of protein probably occurs in extending cells and is independent of cell division. The deposition of additional dry matter in extending cells is no doubt partly due to the formation of additional wall material, which a number of workers have shown does occur in cell extension (see Heyn 1940), and Frey-Wyssling and

Blank (1940), and Blank and Frey-Wyssling (1944) have already shown that an increase in protein nitrogen accompanies the extension of the coleoptile of maize and of the hypanthium of *Oenothera*. But the present series of data also show that the increases in dry matter and protein are exponential, and proceed in a system where fresh weight also increases exponentially.

In the first phase the increases in protein and dry matter are, relative to fresh weight increase, lower than they are in the second. The difference does not necessarily indicate that extension in the first phase is accompanied by a correspondingly less extensive formation of non-labile cellular components. The seedling contains reserves which are no doubt mobilized for the formation of additional wall and protoplasmic materials, and the conversion of these would not of course affect the changes in total protein or of dry matter.

The close connexion between protein, total nitrogen, and growth in the attached seedling, and between protein and growth in the isolated seedling, implies a corresponding connexion between total soluble nitrogen and growth in both. But the data of Table V show that the composition of the total soluble fraction changes with time, and that it is only changes in certain of the fractions that can be related to the growth of the system. In the interpretation of the data of Table V the assumptions are made that the residual soluble fraction represents nitrogen mostly in a peptide-like form, that in the formation of protein amino-acids condense first to form the constituents of the residual nitrogen fraction, and that these subsequently condense to form proteins; that the reverse order of changes occurs in hydrolysis, and that nitrogen is absorbed from the endosperm in the form of amino-acids. For none of these do the present series of data provide conclusive evidence; nevertheless, the scheme for protein formation and hydrolysis suggested is the simplest with which the present series of data can be interpreted, and since neither ammonia in quantity nor nitrates are formed in the seedling, absorption through substances simpler than amino-acids is excluded, and the absorption of more complex substances is in any event improbable.

The composition of the total soluble fraction may be discussed primarily in terms of residual soluble nitrogen, and total amino nitrogen, the non-amide and amide amino groups being treated as components of total amino. In the isolated seedling the hydrolysis of protein leads to an increase in residual soluble and total amino nitrogen between 36 and 60 hours (Fig. 5). But total amino also increases between 2 and 36 hours and between 60 and 96 hours when protein does not change. These increases evidently occur at the expense of residual soluble nitrogen, which decreases slightly during these periods. The factors that control these changes are probably different in the two separate periods. After 48 hours in the isolated seedling no further growth changes occur (Table I), and the increase in total amino in the final period cannot therefore be attributed to this factor. On the other hand, the content of non-amide amino nitrogen is falling rapidly during this time, and if the soluble fractions tend to establish equilibrium concentrations with respect to each other, then the hydrolysis of constituents of the residual

soluble fraction may be determined by the level of non-amide amino nitrogen. In the period between 2 and 36 hours growth is occurring, and the increase in total amino nitrogen is probably an aspect of the changing developmental state.

In the attached seedling the connexion between growth and the levels of the primary constituent of the total soluble fraction is more clearly defined. Residual soluble nitrogen changes in two phases corresponding to those of

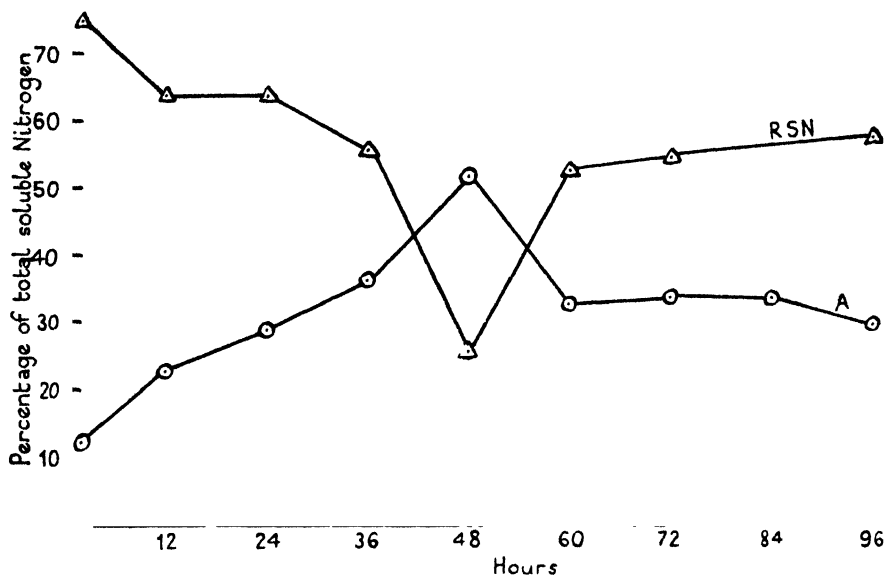


FIG. 7. Proportions of residual soluble nitrogen (RSN) and of total amino nitrogen (A) in total soluble fraction at successive stages of germination in attached seedlings.

growth; in the first it tends to remain more or less constant, and in the second it increases. Total amino nitrogen, on the other hand, increases rapidly and continuously without any well-marked change in the rate of increase throughout the experimental period. These differences imply considerable changes in the proportional contribution of each component to the total soluble fraction, shown by the curves of Fig. 7. During the first 48 hours the proportion of residual to total soluble nitrogen and of total amino to total soluble nitrogen increases and decreases respectively. During the last 48 hours there is a considerable change in the position, the proportion of residual to total soluble nitrogen becomes greater than the corresponding value involving total amino, and during this phase the proportion of each to total amino remains constant. Evidently in the first phase the absorption of amino-acids is accompanied by a relatively slow formation of residual soluble nitrogen constituents, but of a relatively rapid formation of protein from the latter, leading to a decrease in the proportion of residual to total soluble nitrogen. In the second phase the rate of amino-acid absorption is greatly enhanced, but

the increase in the proportion of residual to total soluble nitrogen indicates that there is an increase in the rate of peptide relative to that of protein formation. Moreover, whereas in the first the relative levels of residual soluble and total amino nitrogen do not reach a state of equilibrium, in the second they probably do.

Thus it is evident that in the attached seedling changes in the levels of total soluble, residual soluble, and total amino nitrogen are closely related to corresponding changes in the developmental state, and that the transition from the first phase of growth to the second is accompanied by profound changes in the metabolic situation. The observations of James and James (1940) on the respiration of the whole seed are consistent with this conclusion, for they find that up to 48 hours the rate of oxygen uptake increases with constant acceleration but that after this time the rate of increase falls off, at a variable rate.

While changes in the content of total amino and of residual soluble nitrogen may be associated with growth in the isolated seedling, and undoubtedly are in the attached, changes in the composition of the total amino fraction are apparently independent of growth. In the isolated seedling both amide and non-amide amino nitrogen increase until 48 hours, but thereafter when no further growth changes are occurring, the level of amide increases and that of non-amide decreases. The respiratory quotient values of Fig. 2 indicate that the isolated seedling is subject to acute carbohydrate starvation and that respiration occurs at the expense of a 'protein' substrate. There is little apparent connexion between respiration and the protein level, but a fairly close correspondence between respiration and the non-amide amino nitrogen level, since both increase from 2 to 48 hours and both decrease from 48 to 96 hours, which suggests that the non-amide amino-acid fraction provides the substrate for respiration. The continued accumulation of amide nitrogen is confirmatory evidence for this conclusion. The accumulation of amides in seedlings from protein-rich seeds has been attributed to the oxidation of amino-acids (Chibnall, 1939), and an increase in the level of amides has been observed in detached leaves in which a carbohydrate shortage has developed (Yemm, 1935).

In attached seedlings non-amide amino increases continuously, whereas sucrose, as indicated by the data of James (1940), decreases during the first 2 days of germination and only then starts increasing. Thus there is a closer correlation between non-amide amino nitrogen and respiration than there is between this and sucrose. Moreover, the changes in the level of amide are most suggestive in this connexion. Amide nitrogen, after a preliminary decrease between 2 and 24 hours, increases slowly between 24 and 36 hours, and thereafter relatively rapidly. These changes may be compared with the changes in the respiration rate for this series, which is more or less constant until 24 hours, increases slowly between 24 and 36 hours, and again rapidly after this time. Thus the amide level follows respiration and not growth, which does not increase markedly until 48 hours. There is one further item

of evidence which suggests that respiration in the attached seedling involves the oxidation of amino-acids, and that the level of the latter controls the rate of the former; it is that the average rate of oxygen uptake per unit non-amide amino nitrogen in the detached and in the isolated seedling is almost the same. The relevant data are given in Table VIII.

TABLE VIII

*Oxygen (mml.) absorbed per mg. Non-amide Amino N per hour by Isolated and Detached Seedlings at Different Stages of Germination*

Age of seedlings (hours).	Isolated.	Detached.
12	2,440	—
24	3,066	851
36	2,133	1,395
48	1,884	2,094
60	1,240	1,595
72	2,783	3,018
84	1,710	—
96	3,066	3,052
Mean 36: 96 hrs.	2,137	2,231

In Table VIII the isolated are not compared with the attached, since the exchange of these is restricted by attachment to the rest of the seed (see above), and the oxygen uptake per unit of metabolic substrate is more appropriately measured immediately after separation from the seed. The values of Table VIII do not of course indicate that the only metabolite involved in the respiratory process is non-amide amino-acid (the relevant respiratory quotient data of Fig. 2 suggest that carbohydrates must also be broken down in the process), but they do provide confirmatory evidence for the suggestion that the level of amide in the attached seedling is the result of the oxidation of amino-acids and is not determined by the growth reactions of the seedling.

With respect to the attached seedling, the whole body of data indicates an abrupt change in the growth rate accompanied by a corresponding change in the metabolic situation between 48 and 60 hours. The change is certainly complete within 12 hours, and the protein and total nitrogen data suggest that it may occur within the remarkably short period of 6 hours. The rapidity of the change is indeed the most striking feature about it and suggests some form of 'trigger-action', depending on the acquisition by the seedling of some particular growth factor. It is possible that particular substances are localized within limited regions of the endosperm and that these are released at definite stages of germination by a regular extension of the hydrolytic process into successive zones. This suggestion, however, is an entirely speculative one. As indicated above, the change is certainly not determined by a corresponding change in the continuity of the seed-coat membranes, since these are broken at least 24 hours before the change occurs, and after this stage there is apparently little further change in the structural relations between the seedling and the rest of the seed.



### SUMMARY

The experimental design includes two cultural series, one of seedlings grown in the normal circumstances of attachment to the rest of the seed, and another of seedlings excised from parent seeds that have been in contact with water for 2 hours. Both series are cultured in the same conditions and in contact with water, the experiments being continued for 96 hours.

For each series data are given showing the change with time in the dry weight, the fresh weight, the gaseous exchange, the levels of total nitrogen, soluble nitrogen, protein nitrogen, residual soluble nitrogen, total amino nitrogen, amide amino nitrogen, and non-amide amino nitrogen.

The fresh weight of the isolated seedling increases rapidly until 48 hours and then remains more or less constant, while that of the attached increases slowly until 48 hours and rapidly thereafter. At 48 hours the fresh weight of the isolated is greater than that of the attached, and this difference is attributed to a higher level of water availability in the first.

The dry weight of the isolated seedling falls during the first 12 hours and again, although more slowly, during the final 84 hours. The preliminary decrease, since it corresponds to simultaneous decreases in total, soluble, residual soluble, and amide-amino nitrogen, is attributed to leaching. The fall during the final period is probably due to respiratory losses.

The dry weight, total nitrogen, and protein nitrogen of the attached seedling increase in two phases, the first being one of slow and the second one of rapid increase. The transition from the first to the second phase occurs between 48 and 60 hours and is probably complete within 6 hours.

In the attached seedling fresh weight increase in the first and second phases, dry weight increase in the second, total nitrogen increase in the first and second, and protein nitrogen increase in the second are all probably exponential.

The exponential increase in each phase and the abrupt rise in the relative rate of increase indicate, it is suggested, that the absorption of nutrients from the endosperm is largely determined by the growth reactions of the seedling itself.

The water-content increases in the isolated seedling up to 48 hours and in the attached throughout the experimental period indicating the incidence of cell extension over corresponding times. The evidence suggests that in cell extension there is a deposition of dry matter and of protein nitrogen, the increases occurring exponentially in a system which is itself expanding exponentially.

In the isolated seedling between 2 and 36 hours and again between 60 and 96 hours amino nitrogen increases by the hydrolysis of constituents of the residual soluble nitrogen fraction; between 36 and 60 hours both residual soluble and total amino nitrogen increase by the hydrolysis of protein. These changes are related to simultaneous changes in the growth process.

In the attached seedling residual soluble nitrogen remains more or less constant between 2 and 48 hours and then increases; while total amino nitrogen increases continuously from the beginning of the experiment. In terms of the percentage of each of these as fractions of the total soluble nitrogen, during

the first phase residual soluble nitrogen decreases continuously and amino nitrogen shows a complementary increase; but after 48 hours the proportion of each in the total soluble fraction tends to remain constant. These changes undoubtedly indicate a profound change in the metabolic situation that coincides with the change in growth rate.

Evidence is presented which suggests that the respiration rate in both series is determined by the level of non-amide amino-acids, and that the accumulation of amide is related to this condition.

Changes in the rate of water uptake and of cell extension do not coincide with changes in the rate of oxygen uptake, and it is suggested that cell extension is not limited by the respiration rate.

The present series of data do not provide any indication as to the origin of the abrupt change in the development of the attached seedling occurring at 48 hours. It is not determined by the breaking of the seed-coat membranes, since this happens at least 24 hours earlier.

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## CORRIGENDA

N.S., Vol. X, No. 37 (January), 1946, article by  
R. F. WILLIAMS, 'The Physiology of Plant Growth'.

p. 46, line 5

*for* that large negative errors may occur not only in  
*read* that large negative errors seldom if ever occur, but  
that large positive errors may occur not only in

p. 53, line 32, *for* (see p. 50) *read* (see p. 10)



# Experimental and Analytical Studies of Pteridophytes

## VII. Stelar Morphology: The Effect of Defoliation on the Stele of *Osmunda* and *Todea*

BY

C. W. WARDLAW

(*Department of Cryptogamic Botany, University of Manchester*)

With Plate II and seven Figures in the Text

### INTRODUCTION

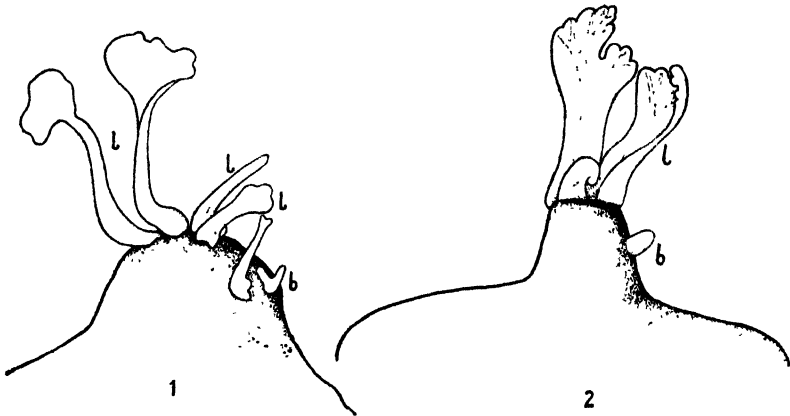
IN leptosporangiate ferns the facts of observation suggest that the conspicuous parenchymatous leaf-gaps in the vascular cylinder of the shoot occur in regions which have been subjected to mechanical stress during development. This stress has been referred to the considerable tangential growth enlargement undergone by the vascular system of the leaf primordium. The hypothesis that a direct relationship exists between leaf development and the internal morphology of the shoot was made the basis of experiments: it was demonstrated in species of *Dryopteris* that, if a succession of very young leaf primordia are destroyed or suppressed, the shoot stele develops not as a dictyostele with wide leaf-gaps, but as an uninterrupted solenostele (Wardlaw, 1944*a*). Such a result also supports the view that in these ferns the shoot stele is essentially of axial or cauline origin and is not merely a composite structure composed of decurrent leaf-traces.

The influence of mechanical factors on the internal tissue pattern during development is clearly a matter that merits the fullest exploration. Accordingly, it seemed cogent to inquire if further experimental verification of the relationship under consideration could be obtained from ferns possessing a rather different type of vascular system. The *Osmundaceae* appeared to afford favourable materials for such investigations. In these ferns the vascular system of the adult shoot consists either of a medullated protostele, as in *Osmunda regalis* and species of *Todea*, or of a special type of solenostele, as in *O. cinnamomea*. On becoming conjoined with the periphery of the shoot stele the crescentic leaf-traces do not produce foliar gaps, i.e. regions of continuous parenchymatous tissue between cortex and pith, as in dictyostelic and solenostelic ferns; but in relation to the insertion of each leaf-trace, a vertically elongated parenchymatous gap is present in the xylem. These xylic gaps correspond in position and development to the foliar gaps in leptosporangiate ferns. If, in the shoot stele of *Osmunda*, stresses arising from the tangential enlargement of the leaf-traces are causally related to the development of the xylic gaps, then, by suppressing the leaf primordia at a very early stage, it

should be possible to produce a continuous, unbroken cylinder of xylem. As in *Dryopteris*, this treatment should also yield data on the extent to which the shoot stele is axial or foliar in origin.

#### MATERIALS AND METHODS

Stout shoots of *Osmunda regalis*, *O. cinnamomea*, and *Todea barbara* were completely defoliated down to the smallest primordia and the scales removed so that the slightly convex shoot apex was left exposed. But whereas in *Dryopteris aristata* this was a comparatively simple operation (Wardlaw,



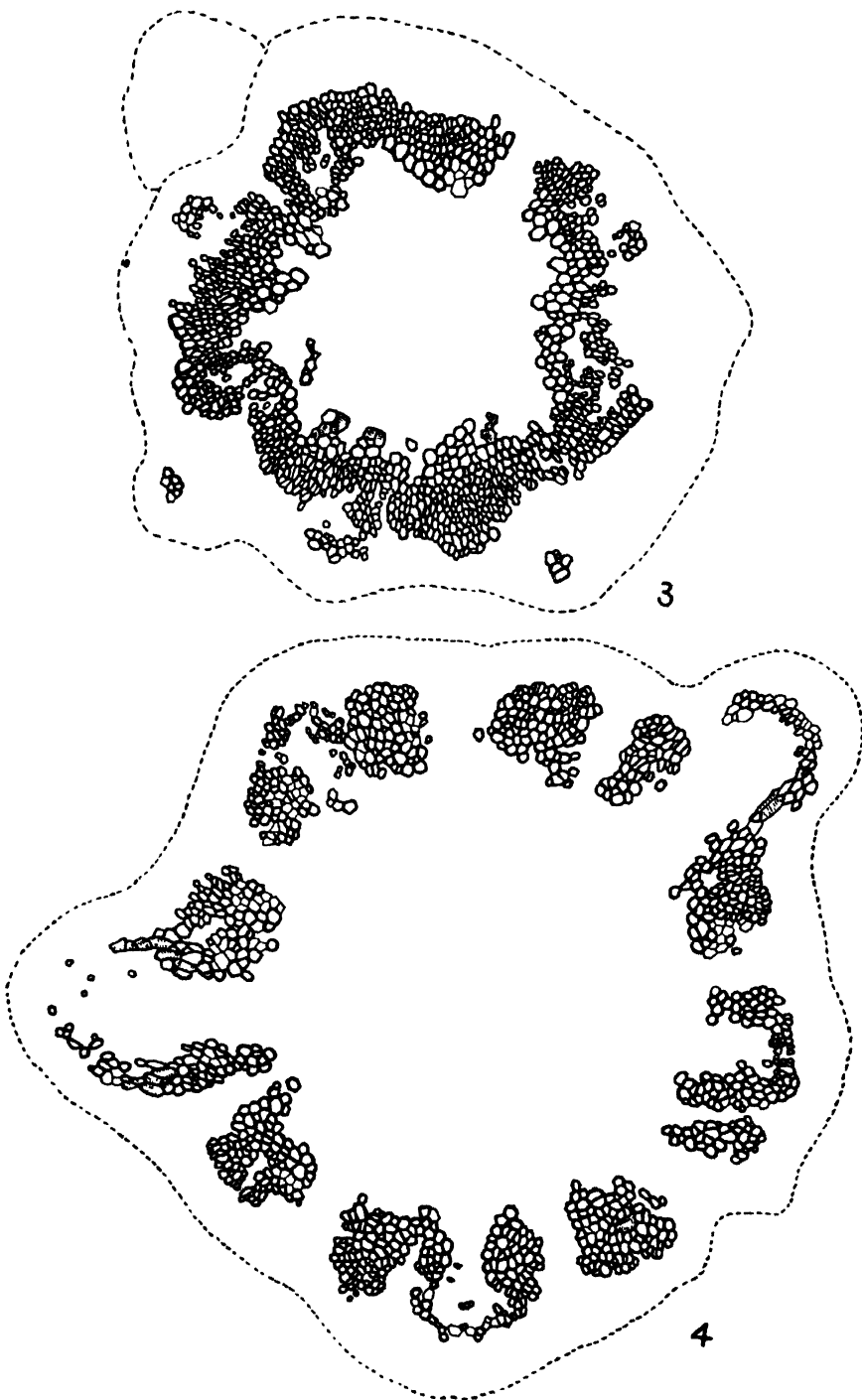
TEXT-FIGS. 1 and 2. Fig. 1. *Osmunda regalis*. Fig. 2. *Todea barbara*: Distal region of plants which had been continuously defoliated, showing terminal group of greatly reduced leaves (*l*) and lateral buds (*b*).

1944a), in *Osmunda* and *Todea* considerable difficulty was experienced. This relates to the fact that in these ferns the apical meristem lies in a sunken position closely over-arched by a compact mass of young leaf primordia and scales. Moreover, the copious secretion of mucilage tends to obscure the view and renders exact dissection difficult. A number of specimens were lost through the inroads of fungi.

The apices were at first protected by means of moist cotton wool, but later this was found to be unnecessary. The pieces of shoot were planted in moist peat in a cool greenhouse and all new primordia removed at weekly or fortnightly intervals. This procedure was discontinued after some time, the last primordia to be formed being allowed to grow on. In the two specimens of *O. regalis* shown in Pl. II, Figs. 1 and 2 and Text-fig. 1, the bulky shoots are terminated by an inconspicuous group of *juvenile* leaves. These materials were fixed and serial sections cut in basipetal sequence.

#### OBSERVATIONS ON THE EFFECT OF DEFOLIATION

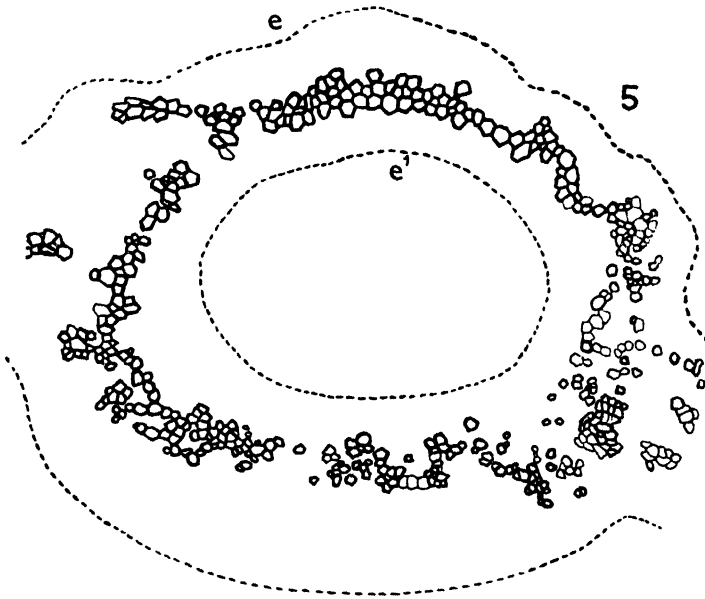
*Osmunda regalis*. In the defoliated region of the shoot in one specimen some of the primordia had been destroyed at such an early stage that little evidence



TEXT-FIGS. 3 and 4. *Osmunda regalis*. Transverse section of shoot in defoliated region (Fig. 3) and in normal region below (Fig. 4). In the former the tracheides form an almost uninterrupted woody ring. ( $\times 65$ .) (Figures from ink tracings over photographs.)

of their existence remained; others had undergone some development before being excised. As Text-figs. 3 and 4 and Pl. II, Figs. 6–8, show, the xylem in the experimental region of the shoot forms an almost continuous ring, the insertion of the occasional arrested leaf-trace having failed, in most instances, to produce a xylic gap, whereas, in the normal region below, many conspicuous xylic gaps are present. In other specimens similar evidence of continuity in the xylem cylinder has been obtained.

*Osmunda cinnamomea*. Text-fig. 5 shows the more or less complete ring of xylem observed in the experimental region of a shoot.



TEXT-FIG. 5. *Osmunda cinnamomea*. Transverse section through defoliated region of shoot, showing the tracheides distributed in a thin almost continuous ring. *e*, outer endodermis; *e'*, inner endodermis. ( $\times 60$ .) (Figure from ink tracing over photograph.)

#### FURTHER OBSERVATIONS ON STELAR MORPHOLOGY

It has been noted that plants of *Osmunda* and *Todea* which had been continuously defoliated eventually developed only juvenile leaves. Points of anatomical interest in these specimens may now be briefly considered.

*Osmunda regalis*. Serial sections of the large experimental shoot illustrated in Pl. II, Fig. 1, yielded the following information. Ten leaves, including leaf primordia, surrounded the apical meristem: the three youngest primordia lay close to the apex; three larger ones, with partly differentiated vascular strands showing one or two tracheides, lay farther out; while the outermost leaves were those of which the traces are shown in Pl. II, Fig. 3. A short distance below the apical meristem the shoot stele was of large cross-sectional area



relative to the small leaf-traces at its periphery. At this level the stele was elliptical in outline and somewhat flattened on one side. The conspicuous feature of the stele was the vascular trace of an awl-like leaf of the second whorl indicated above. This leaf-trace had well-developed xylem which passed right into the pith of the shoot stele and terminated in a series of large, well-lignified tracheides, Pl. II, Fig. 3. About this level, groups of metaxylem tracheides of axial origin also began to be present, not only in the normal metaxylem region but also in the pith; at the periphery the small crescentic xylem masses of the leaf-traces were to be seen, Pl. II, Figs. 3, 4. On proceeding down the shoot the amount of axial xylem increased. This was of two categories: many large and conspicuous tracheides were scattered through the pith, producing an effect not unlike the 'mixed pith' described by Gwynne-Vaughan (1914); other, smaller, tracheides, occupying the metaxylem position round the pith, formed a more or less continuous ring to which the decurrent xylem of the leaf-traces became conjoined. It was thus possible to observe the contributions made by the leaf-traces and the shoot to the common stele, vascular tissue of axial origin being by far the more conspicuous.

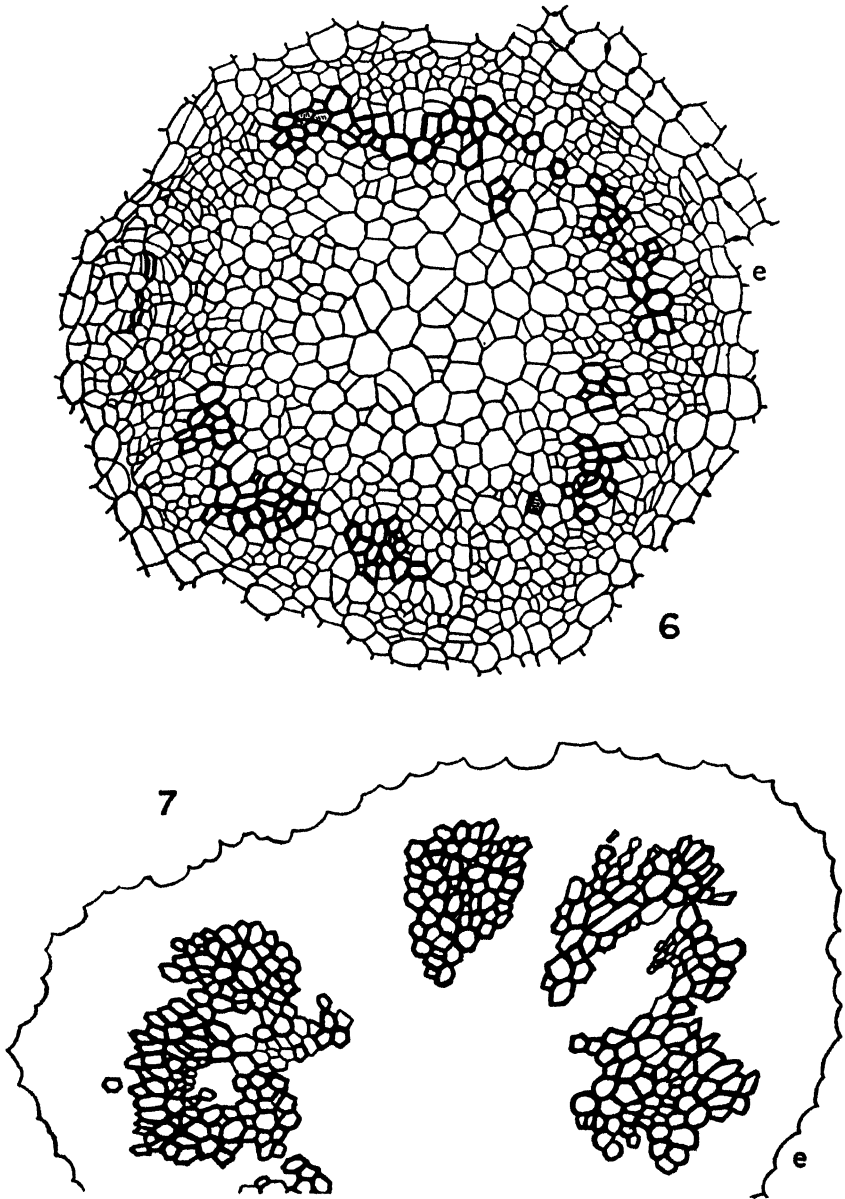
Farther down the shoot, in the region of defoliation, the 'mixed pith' was replaced by normal parenchymatous pith, and the xylem, as already described, consisted of an almost continuous woody ring. Only one specimen showed the presence of tracheides scattered throughout the pith. In *O. cinnamomea* Faull (1909) has called attention to the presence of groups of tracheides in the pith in the region of bifurcation.

Text-fig. 1 and Pl. II, Figs. 1, 2, show that the experimental treatment adopted led to the production of leaves which were greatly reduced in size and in structural complexity. They were, in fact, of the type described as 'juvenile' and were closely comparable in size and venation with the first leaves of the young sporophyte. This conspicuous reduction in leaf size is evidence that the normal growth processes have been arrested or modified, but the factors specifically involved cannot be decided on the evidence yet available. Such conspicuous evidence of arrested growth, sometimes described as regression, as that shown in Text-fig. 1, where the leaf is merely an awl-like outgrowth, is of particular interest in the study of morphogenetic processes. Thus the small, simple leaf of the sporeling and the large, complicated leaf of the adult are seen to arise, under different conditions of growth, from equivalent primordia.

*Todea barbara*. After a period of continuous removal of all leaf primordia, a large shoot of this species was allowed to grow on. A group of greatly reduced leaves developed, Text-fig. 2. At this stage the specimen was fixed and sectioned.

As a result of the experimental treatment the shoot stele underwent a great reduction in size, the partly differentiated stele below the apex being not unlike that of a young plant. The first tracheides to be differentiated in the terminal region of the shoot, Pl. II, Fig. 9, were those associated with the decurrent leaf-traces of the leaves shown in Text-fig. 2. Lower down, in the defoliated

region, leaf-trace xylem was absent from the periphery of the stele, the meta-xylem consisting of an incomplete thin ring of tracheides, Text-fig. 6. At one



TEXT-FIGS. 6 and 7. *Todea barbara*. Fig. 6, reduced stele in defoliated region of the shoot. Fig. 7, distribution of xylem in normal region of shoot below. *e*, endodermis. ( $\times 125$ .) (Figures from ink tracings over photographs.)

level a very small inner stele, lying excentrically in the pith, made its appearance but soon tapered out, Pl. II, Fig. 10. Such abnormalities are of interest

in that they disclose potentialities of the growing region not usually evident during 'normal' development. Still lower down in the experimental region, where the stele was of approximately adult diameter, the xylem consisted of a thin, incomplete ring of metaxylem. Here, as in *O. regalis*, the axial nature of the shoot stele is demonstrated. In the normal region of the shoot below, the distribution of the xylem in conspicuous broad wedges (Text-fig. 7) was in marked contrast to the condition observed in the defoliated region.

#### DISCUSSION

The evidence obtained from species of *Osmunda* shows that, if young leaf primordia are suppressed, xyletic gaps are not formed in the shoot stele, and the xylem develops as an uninterrupted woody cylinder surrounding a central pith. The experimental data also justify the view of Bower (1923, p. 139) that the normal shoot stele is not merely a structure built up by the fusion of decurrent leaf-traces: it is, in fact, a composite structure in which vascular tissue of axial and foliar origin can be recognized and separated by experimental means. Thus a stele can be produced in which conducting tissue of foliar origin is more or less completely lacking. In such a stele the radial extent of the woody tissue tends to be less than in the normal stele. These experimental observations and the discussion based on them are capable of further extension. Because of the limited amount of material available for study, the further inferences which might be drawn will be held over until later. It may, however, be noted that the data support the hypothesis that the initial differentiation of vascular tissue takes place in a basipetal direction in relation to growth processes at the apices of shoot and leaves (Wardlaw, 1944). Some further experimental possibilities may perhaps be indicated here. In different species of *Osmunda* and *Todea* it may be expected that the respective foliar and axial contributions to the shoot stele may vary, and hence, under experimental treatment of the type described here, steles of somewhat different appearance may be obtained. In this connexion the variable position of the protoxylem in the normal shoot stele of different species is of interest. The protoxylem is situated on the adaxial side of the leaf-trace and therefore when the latter becomes completely confluent with the shoot stele, the position of the protoxylem will show to what extent the shoot stele is of foliar origin.

The number of xylem strands varies greatly in different species (Faull, 1901, 1909). Thus *O. claytoniana* may have as many as 40, *O. regalis* about 15, *Todea barbara* about 8, while in *T. superba* the xylem may sometimes appear as an unbroken cylinder. The number of xylem strands observed in any transverse section depends on the number of xyletic gaps—the parenchymatous tissue of which constitutes the so-called medullary rays extending from pith to phloem. The number of xyletic gaps present at any level depends on several factors including the phyllotaxis, the vertical extent of the gap, and the length of the internodes. The xyletic gaps in *T. barbara*, for example, appear

to be considerably shorter than those of *Osmunda regalis* (Kidston and Gwynne-Vaughan, 1907); hence, if the phyllotaxis and length of internode were approximately the same in both, then the former would show fewer xylem strands in a transverse section than the latter. The length and width of the xylic gap may tentatively be referred (*a*) to the intensity of the stress in the developing shoot stele produced by the tangential enlargement of the leaf-base, and (*b*) to the longitudinal growth of the shoot. The nexus of factors involved is clearly a complicated one, but a study of the distribution of growth at the shoot apex, including the leaf primordia, seems likely to yield data relating directly to the structural developments under consideration.

If, as the evidence suggests, mechanical stresses are important in stelar development, it may be inferred that the rate of tangential growth of the leaf-trace relative to the rate of peripheral extension of the shoot stele will be one of the factors which determine whether or not foliar or xylic gaps are produced. In this connexion further investigation both of living species and of the fossil Osmundaceae (Kidston and Gwynne-Vaughan, 1907-14) seems likely to be productive of interesting results. The matter cannot be discussed in detail here, but it may be noted in passing that in some of the larger fossils (e.g. *Thamnopteris Schlechtendalii* Eich), in which the leaf-traces are of small size relative to the shoot stele, the latter is characterized by the presence of a massive uninterrupted cylinder of xylem. Sinnott (1910), however, has recorded his view that in all the fossil Osmundaceae which possessed a true parenchymatous pith the departure of a leaf-trace was attended by a gap in the xylem ring. In this connexion Tansley (1907-8) has pointed to the importance of the size-relation of leaf-trace to shoot stele.

The presence of tracheides scattered through the pith, giving rise to the condition described as 'mixed pith', has previously been reported by Gwynne-Vaughan (1914) for *Osmunda regalis* and by Bower (1911) for *Botrychium ternatum*. In these instances it is believed to follow on traumatic injury. The presence of tracheides in the pith has been used in support of the view that phylogenetically the pith represents a modified xylem core, i.e. 'that it is a consequence of a change of procambial destination of the central tract of the xylem from development as tracheides to development as parenchyma. The isolated tracheides are then held to be residual cells in which the change has not been perfectly carried out, or has been for some reason reversed' (Bower, 1923, p. 126). However that may be, it may be noted that injuries, like those produced by the method of dissection used here, tend to modify the system of tensions and compressions within the normal growing shoot, and therefore to affect the differentiation of the component tissues.

The present studies have produced materials of considerable interest to the morphologist in that they illustrate both regression in leaf development and reduction in the vascular system. Goebel (1900) held that the primary leaves of the young sporophyte are strictly comparable in origin and structure with adult leaves but that their development has been arrested—a view which is

supported by the data presented here. He has further observed that the construction of the primary leaf of the young sporophyte varies and that the higher or more elaborate form is more rapidly attained in robust sporelings. He has also shown that a regression in leaf development can be induced when certain ferns are grown under unfavourable conditions. In *Ceratopteris thalictroides* regression was induced by culturing isolated stem apices (1908); the first leaves produced by the isolated tips were of the juvenile type, the degree of regression being correlated with the age of the plant. As in the previous experiment, the evidence suggests a correlation between the amount of nutritive material available and the leaf form developed. Notable changes in leaf development in *Onoclea sensibilis*, associated with growth under unfavourable conditions, viz. high temperature, high humidity, and feeble illumination, have also been recorded (Wardlaw, 1945): regression from adult to juvenile leaves, and from the latter to non-laminate awl-like organs was observed. Far-reaching changes in the internal structure of the shoot accompanied these external evidences of 'starvation' conditions at the apex. It may be noted that although the growth of both shoot and leaves was on a greatly reduced scale, abundant deposits of starch were present in all cortical and medullary tissues. Theories which refer regressive changes to 'starvation' conditions, or in general terms to insufficiency of metabolites in the growing region, must be viewed with caution until fuller information has been obtained.

The plants of *Osmunda regalis* and *Todea barbara* described here afford further evidence of regression. In some instances leaves have been reduced to simple awl-like outgrowths, very like those observed by Lang (1924) in induced lateral buds. In *T. barbara* the vascular system of the shoot in association with the juvenile leaves showed a marked reduction both in size and complexity, and was closely comparable with the steles of sporelings investigated by Faull (1901), Seward and Ford (1903), Gwynne-Vaughan (1911), and Cribbs (1920). Thus, in the growth of the individual plant, the normal upgrade development with its progressive increase in structural complexity—the basis of the Theory of Recapitulation—can be reversed by appropriate experimental treatment. The metabolic factors involved require investigation. The changes in structural complexity which are associated with changes in size in these materials support the views of Bower (1930) on the importance of size as a factor in stelar morphology.

Both de Bary (1888) and Van Tieghem (1891) considered that the central cylinder of the Osmundaceae resembled that of the phanerogams; de Bary regarded the vascular system as consisting of collateral bundles of the dicotyledon type; Van Tieghem, too, referred to the presence of bundles, but considered that the vascular system was essentially monostelic. In the light of more recent investigations and of the experimental observations presented here, further discussion of this aspect, as also of the stelar theory of Jeffrey (1897, 1902, 1908)—in which the pith is considered to be an 'inclosed portion of the fundamental tissue'—seems unnecessary.

## SUMMARY

1. On the destruction of a succession of young leaf primordia in *Osmunda regalis* and *O. cinnamomea* the normal dictyoxyllic stele is replaced by one in which the xylem consists of an uninterrupted cylinder of tracheides surrounding the pith.

2. The results obtained support the view that the normal shoot stele is a composite structure including vascular tissues of both axial and foliar origin.

3. Other observations relating to the occurrence of a tracheidal ('mixed') pith in *O. regalis* and of an inner stele, of regression in leaf development, and reduction in stelar complexity in *Todea barbara*, are described and discussed.

The writer has pleasure in acknowledging the assistance received from Mr. E. Ashby in microscope preparations and photographic illustrations.

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## DESCRIPTION OF PLATE II

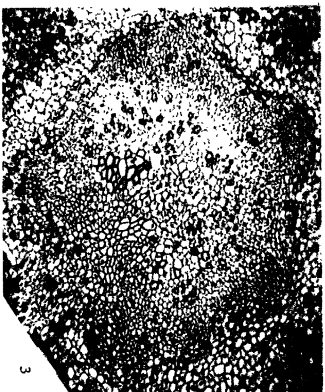
Illustrating Professor C. W. Wardlaw's paper on 'Osmunda and Todea'.

All figures are from untouched photographs.

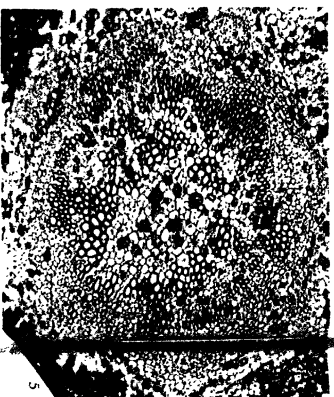
FIGS. 1, 2, *Osmunda regalis*. Experimental plants showing the thick leafy shoots which had been defoliated and the group of small terminal leaves (nat. size).

FIGS. 3-8, *O. regalis*. Series of transverse sections, in basipetal sequence, of the experimental plant in Fig. 1. Fig. 3. Below apex, showing several leaf-traces at the periphery of the stele; the conspicuous group of tracheides in the pith are also of foliar origin (see text), the other scattered tracheides being of axial origin. Fig. 4. A little lower down in the shoot: the xylem of the several peripheral leaf-traces has now moved further into the stele; that of the leaf-trace in the pith is no longer present, but large tracheides of axial origin are now scattered through the pith (giving the condition known as 'mixed pith'); other, smaller tracheides are distributed as an incomplete ring round the pith. Fig. 5. Still lower in the shoot, just above the defoliated region: a conspicuous 'mixed pith' is now present, and also an incomplete ring of axial tracheides round the pith. Fig. 6. Section through the defoliated region: only occasional tracheides are present in the pith; the tracheides now form an almost continuous woody ring; no leaf-traces are present at the periphery of the stele, though the xylem of leaves inserted higher up is becoming confluent with that of the shoot stele. Fig. 7. Section taken near the lower limit of the defoliated region: the continuous ring of xylem is interrupted by some gaps relating to leaves inserted lower down. Fig. 8. Section through normal region of shoot below, showing the insertion of leaf-traces on the periphery of the shoot stele and the presence of numerous foliar gaps in the xylem. ( $\times 65$ .)

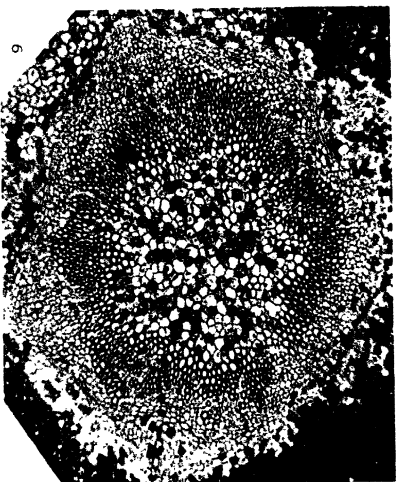
FIGS. 9 and 10, *Todea barbara*. Fig. 9. Small, reduced stele in the terminal region of the experimental shoot illustrated in Text-fig. 2: the groups of tracheides are of foliar origin. Fig. 10. Lower down in the shoot, about the upper limit of the defoliated region: the xylem of two leaf-traces can be seen at and near the periphery of the stele; the axial xylem consists of an interrupted ring of large tracheides; a small inner stele is present in the pith in an excentric position. ( $\times 125$ .)



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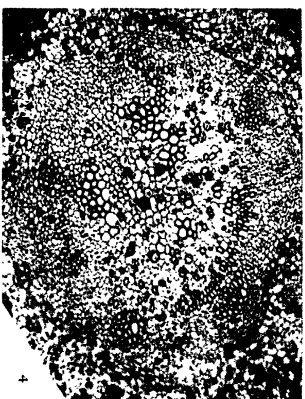


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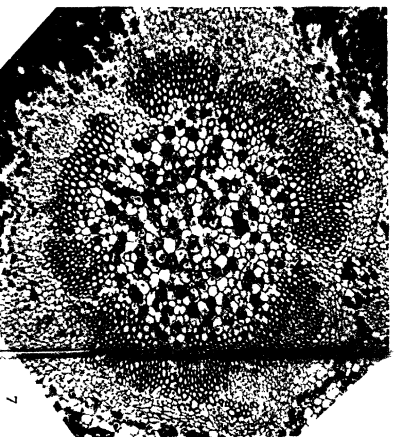


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9



8





# A Cytological Basis of Sterility in *Tripsacum laxum*

BY

K. S. DODDS

AND

N. W. SIMMONDS

(Department of Botany, Imperial College of Tropical Agriculture, Trinidad, B.W.I.)

With Plate III and seven Figures in the Text

## I. INTRODUCTION

GUATEMALA grass is usually referred to *Tripsacum laxum* Nash and this name is retained here, since Cutler and Anderson (1941) do not seem to give adequate reasons for the preference given to *Tripsacum fasciculatum* Trin. ex Asch. It is regarded by Paterson (1939) as a most valuable fodder grass under Trinidad conditions. Usually treated as a soiling grass, and as such cut every 8 to 10 weeks, it is not normally allowed to flower. A plot in St. Augustine, Trinidad, B.W.I., uncut since February 1944, came into flower in late November of the same year, some 2 months before the end of the wet season. Field observation showed that anthesis did not occur and that the anthers were empty though well-formed. Full emergence of apparently normal styles took place. A similar failure of anthesis was noted by Mangelsdorf and Reeves (1939) in certain sterile hybrids in the Maydeae.

Unfortunately this plot was cut before any observations could be made on female fertility. However, the failure of anthesis and the meiotic irregularities to be described below make it virtually certain that no viable seed would have been set. Notes were made on a second large plot of the grass abandoned at River Estate, near Port of Spain. All the inflorescences, which were formed in large numbers, appeared to be completely sterile though the rachis shattered readily: extensive tests, however, failed to yield any seedlings. This sterility is in accord with that reported in Mexican and Guatemalan material by Cutler and Anderson (1941). Longley (1924), however, states that in a greenhouse he raised plants from seed to flowering.

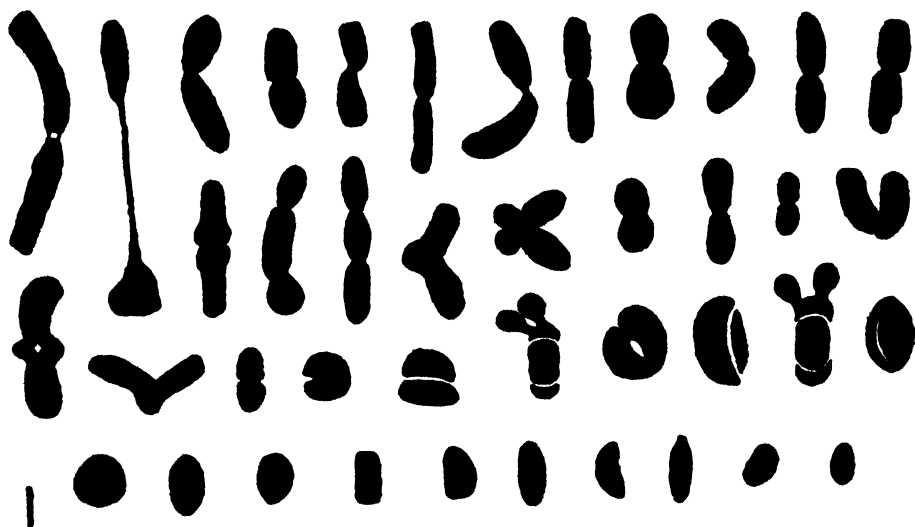
The origin of the material at St. Augustine is uncertain, but most probably it is all clonal. The same type of variation in leaf and panicle as that described by Cutler and Anderson (1941) was seen, indicating that such variation is purely phenotypic and not genetic as these authors suggest.

Meiotic stages were found shortly after the emergence of the inflorescence. Material from one stem was fixed in acetic-alcohol for 24 hours and stored in

70 per cent. alcohol until needed. All preparations were stained in iron-acetocarmine, squashed and made permanent in euparal by the method of McClintock (Darlington and La Cour, 1942). Root-tips obtained from cuttings rooted in sand were fixed in 2BD (Darlington and La Cour, 1942), embedded, cut at  $15\ \mu$  and stained by the gentian-violet-iodine method.

## 2. CHROMOSOME NUMBER

Longley (1924) concluded from a brief study of meiosis that  $2n = 70$ . Mangelsdorf and Reeves (1929) give  $2n = 72$ , based on root-tip counts and



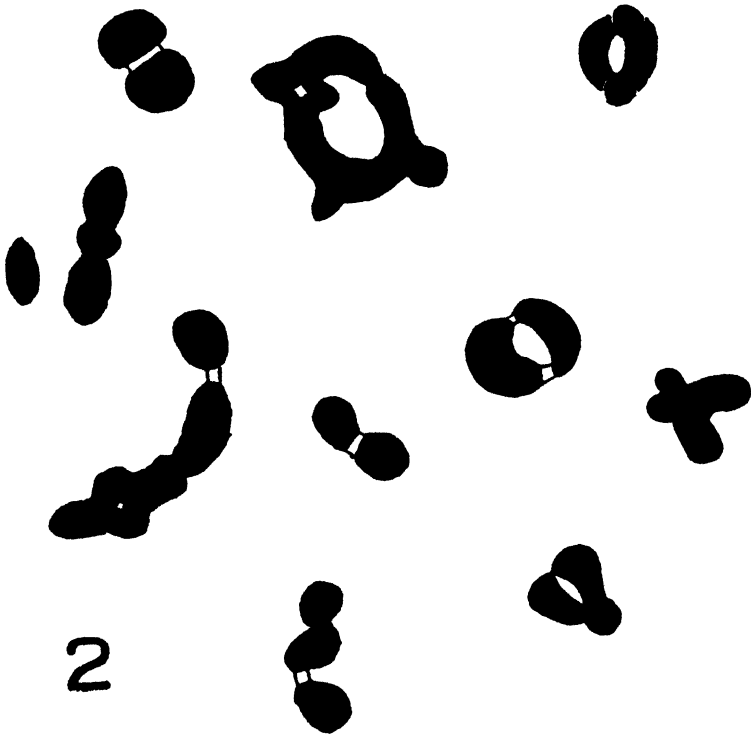
TEXT-FIG. 1. Diakinesis: the complete complement of one cell showing 31 bivalents and 10 univalents. ( $\times 3,300$ .)

state that there was some uncertainty as to the precise number. Root-tip metaphases in the present study were excellent as regards fixation and staining, but it was found impossible to analyse them on account of the dense crowding of the plates and the variability in chromosome size. The longest somatic chromosomes (about  $5\ \mu$ ) were found to be about 5 times as long as the shortest. The most that can be said is that the number was about  $2n = 70$ . Meiotic studies (see below, and Text-fig. 1) indicate  $2n = 72$  with reasonable certainty.

## 3. MEIOSIS

*Prophase.* The chromosomes were far too numerous to permit the analysis of early prophase stages. At pachytene occasional univalents could be distinguished lying apart from the rest of the chromosomes which formed a dense tangled mass of threads. Diakinesis (Pl. III, Fig. 1) offered the best chance of a complete analysis of association, although even here a completely critical

analysis was difficult; but the most satisfactory cell (Text-fig. 1) admitted of very little doubt. The pollen mother cells contained from 10–12 univalents, 25–30 bivalents, and about 2 multivalents. Bivalents with a single terminal chiasma predominated, while of the multivalents, chain forms were the most common (Text-fig. 2). The meiotic complement (Text-fig. 1) showed the



TEXT-FIG. 2. A selected group of diakinesis configurations. There are present 2 quadrivalents, 1 trivalent, 7 bivalents, and 1 univalent with variously arranged chiasmata. ( $\times 3,900$ .)

same great variation in size of individual chromosomes as was noted in somatic mitosis.

*First metaphase.* The metaphase plate was densely crowded and quite impossible to resolve (Text-fig. 3, and Pl. III, Fig. 2). Non-congressed bivalents and univalents were seen in every cell. In addition 10 cells out of 57 showed one and 1 showed two non-congressed trivalents (Table I). These non-congressed elements were mostly polar in distribution relative to the metaphase plate and unoriented (Text-fig. 3 and Pl. III, Fig. 2). According to Darlington (1937, p. 525), non-congression may be a consequence of a crowded plate.

*First anaphase.* Lagging bivalents and univalents were constantly present (Text-fig. 4, and Pl. III, Fig. 3 and Table II). In the case of the former,

complete non-disjunction was common. Dividing univalents were not certainly identified owing to difficulties of interpretation, as a small dividing element could have been either a small bivalent undergoing late disjunction or a large univalent dividing prematurely, such was the range in chromosome size. The polar groups appeared to pass into the scattered elements which had failed to congress and of which some were included in the telophase nuclei (Text-fig. 4 and Pl. III, Fig. 3).

TABLE I

*Non-congression at First Metaphase. Numbers of First Metaphases having Various Numbers of Non-congressed Bivalents and Univalents. The sign III indicates a cell having a trivalent together with the appropriate number of bivalents and univalents. The mean is 5.3 univalents and 5.1 bivalents per cell.*

		Bivalents										
		1	2	3	4	5	6	7	8	9	10	11
Univalents	2	—	1	1	—	1	1	—	—	—	—	—
	3	—	1	—	—	2	+	—	1	—	—	III
	4	—	—	2	1	1	1	1	2	—	—	+
	5	—	—	1	3	3	1	1	1	1	1	—
	6	1	—	+	+	—	+	1	+	—	—	—
	7	III	—	—	—	—	III	—	—	—	—	—
	8	+	1	—	—	—	—	1	2	—	—	—
	9	—	—	—	—	—	—	1	—	—	—	—
		* Two trivalents present.										
											Total 57	

TABLE II

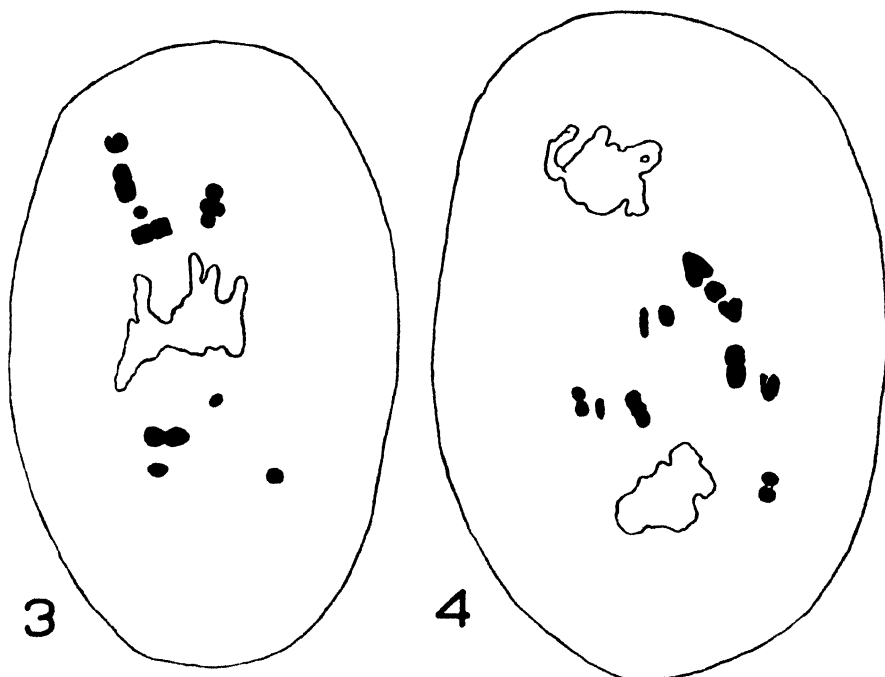
*Irregularities of Disjunction at First Anaphase. Numbers of First Anaphases having Various Numbers of Lagging Bivalents and Univalents. The mean<sup>\*</sup> is 2.5 univalents and 3.4 bivalents per cell*

		Bivalents								
		0	1	2	3	4	5	6	7	8
Univalents	0	—	—	—	—	—	1	1	—	—
	1	—	1	—	—	1	—	—	—	—
	2	—	—	—	2	1	—	—	1	1
	3	—	—	1	—	—	—	—	—	—
	4	1	1	—	—	—	—	—	—	—
	5	—	1	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—
	7	—	—	1	—	—	—	—	—	—
										Total 14

Comparing Tables I and II, it will be seen that a mean total of 7.8 univalents per cell is accounted for during metaphase and anaphase. The observa-

tion of 10–12 univalents per cell at diakinesis suggests that some were lost to analysis either by early division or by passive inclusion in an anaphase group.

*Interphase.* There was a well-marked interphase in which some despiralization took place and, in some cells at least, a nucleolus was organized. Chromosomes lagging from anaphase and those which failed to congress at metaphase



TEXT-FIGS. 3 and 4. Fig. 3. Metaphase I with 5 bivalents and 4 univalents which have failed to congress. ( $\times 1,600$ .) Fig. 4. Late anaphase I with 7 lagging or non-congressed bivalents and 4 univalents. ( $\times 1,600$ .)

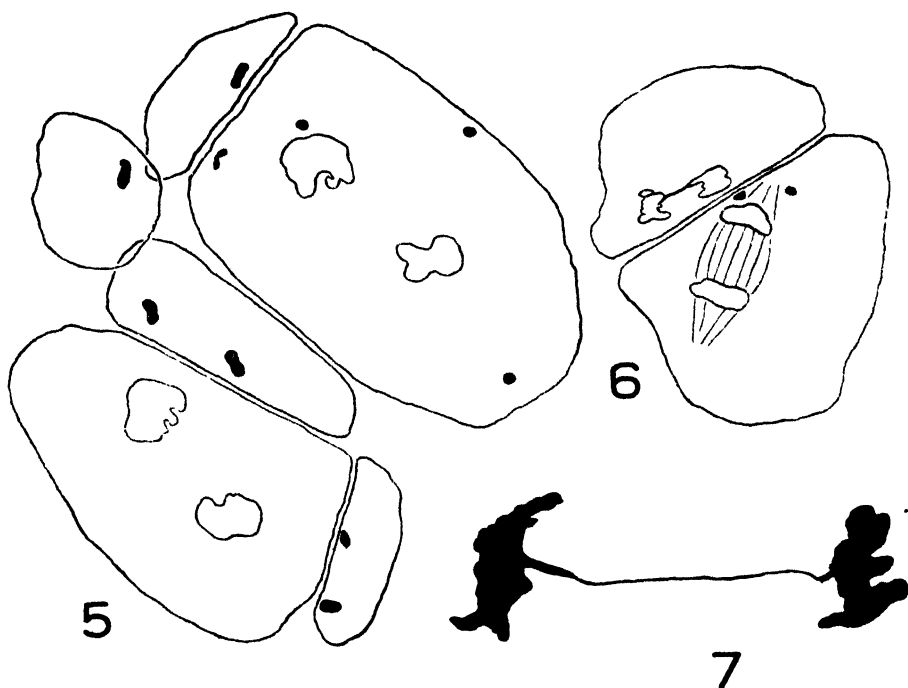
an dwere not included in the subsequent anaphase movement were seen to (1) form micronuclei, or (2) remain free and condensed in the cytoplasm (cf. Myers and Hill (1942) on *Dactylis*), or (3) form microcytes, particularly if lying in a more or less polar position (Text-figs. 5, 6, and Pl. III, Fig. 4). The component cells of the interphase diad separated easily so that the data for microcyte frequency given in Table III may be a slight underestimate as a result of loss.

TABLE III

*Irregularities at Second Division. Numbers of Cells having Various Numbers of Microcytes per Diad, Non-congressed Chromosomes at Second Metaphase, and Laggards at Second Anaphase*

	0	1	2	3	4	5	Totals	
Microcytes per diad	13	23	14	3	1	0	54	} Nos. of cells
Non-congression at second metaphase	22	23	18	10	5	2	80	
Laggards at second anaphase	6	4	2	0	0	0	12	

*Second division.* Second metaphase plates were markedly more regular in appearance than those of first metaphase. Nevertheless, failure of orientation was frequent (Table III and Pl. IV, Fig. 4). Irregularities at second anaphase included laggards in about 50 per cent. of the cells (Table III) and occasional bridges without fragments (Text-figs. 6 and 7). One case of a bridge and laggard in the same cell was seen, and one case also of a double spindle. Most



TEXT-FIGS. 5-7. Fig. 5. Telophase 2 in a 'diad' with 4 microcytes. Two of the microcyte nuclei have evidently divided, two have not. Note the tendency to early rounding off and separation of the component cells. ( $\times 1,500$ .) Fig. 6. Anaphase 2 in one cell of a diad showing non-congression and a bridge in the dividing microcyte nucleus. ( $\times 1,500$ .) Fig. 7. Second telophase showing bridge. ( $\times 2,100$ .)

microcytes divided (Text-figs. 5 and 6). Sometimes a slight lack of synchronization between the cells of a diad occurred so that anaphase movement began in one cell before the other. This same lack of synchronization was seen occasionally at second telophase, when one pair of nuclei despiralized and organized a nucleolus more rapidly than the other.

Bivalents remaining undivided from first division (i.e. either previously non-congressed or laggards) were not certainly identified at second division. The second metaphase plates appeared compact and regular without the protruding arms that might have been expected had there been bivalents present. Nor, judging by size, were bivalents present free in the cytoplasm or in microcytes. It therefore seems probable that those which remained undivided from first division disjoined during interphase; though it must be emphasized that

the evidence on which this view is based is not critical. Darlington (1929) found a similar behaviour in a triploid *Hyacinthus*, and he states (1937) that disjunction is general where an interphase occurs.

The component cells of the 'tetrads' rounded off rapidly and gave rise to very irregular pollen grains. At maturity the small amount of pollen in the anther was ill formed and appeared inviable.

#### 4. DISCUSSION

The material of *T. laxum* described here is evidently a tetraploid (cf. Mangelsdorf and Reeves, 1939) and suffers the sterility consequent on irregular association, crowded metaphases, and irregular disjunction. A successfully reduced gamete must be of very rare occurrence. It may be inferred that the sterile material of Cutler and Anderson (1941) is cytologically similar. As, in general, there is a higher survival of aneuploid spores in the female than in the male, given a fertile male parent, occasional viable seed might be obtained by crossing and a beginning made towards the selection of a fertile line for breeding purposes.

The high bivalent frequency perhaps suggests allopolyploidy (amphidiploidy), and this view is supported by the greater regularity of association found by Longley (1924) in material from Salvador. However, a low chiasma frequency in an auto-tetraploid might well account for the observed associations. A decision must await a comprehensive cytogenetic investigation of the whole genus.

A most interesting cytological feature is the presence of bridges at second anaphase. Richardson (1936) has reviewed the consequence of various structural changes in relation to crossing-over, and has shown that bridges at second anaphase may arise from inversions, duplications, and translocations, but that in every case these same structural changes will produce bridges with or without fragments at first anaphase. Moreover, the arrangement of chiasmata necessary to produce bridges at second anaphase is more complex than that required to produce bridges at first anaphase. In other words, the occurrence of second division bridges, in their absence from first division, renders it unlikely that any ordinary structural change is responsible. This is precisely the situation found in the present material. The only possible explanation would seem to be fusion of the homologous ends of sister chromatids prior to second division. Such fusion has been inferred by Upcott (1937) for pollen grain mitosis in *Hyacinthus orientalis* and by Barber (1938) for tube mitosis in old pollen grains of *Kniphofia rufa*. A similar behaviour appears to occur in the second division of *Crotalaria incana* (unpub.). Darlington and Upcott (1941) have reviewed the whole situation and give further examples.

#### 5. SUMMARY

The chromosome number of *Tripsacum laxum* Nash was confirmed as  $2n = 72$ . Most chromosomes were associated as bivalents but there were



irregularities of association, congression and separation resulting ultimately in male sterility.

Bridges were not seen at first anaphase: their occasional presence at second anaphase was interpreted as a consequence of chromatid fusion.

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#### DESCRIPTION OF PLATE III

Illustrating Dr. K. S. Dodds and Mr. N. W. Simmonds's article on A Cytological Basis of Sterility in *Tripsacum laxum*.

FIG. 1. Diakinesis. (*c.*  $\times$  1,500.)

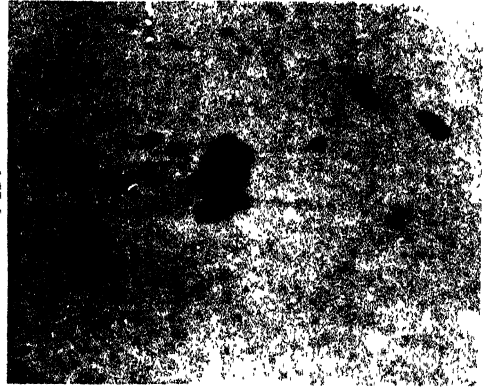
FIG. 2. First metaphase with non-congressed univalents and bivalents (*c.*  $\times$  1,500.)

FIG. 3. First anaphase with laggards. (*c.*  $\times$  1,500.)

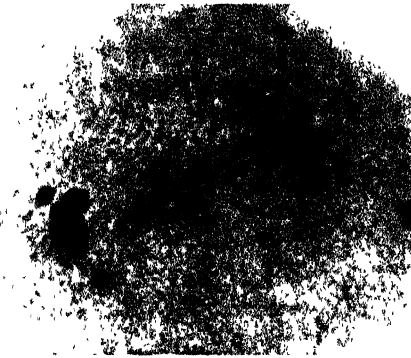
FIG. 4. Second metaphase diad with microcyte and a non-congressed chromosome in one cell. (*c.*  $\times$  1,000.)



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2



3



4

Huth coll.

DODDS & SIMMONDS — *TRIPSACUM LAXUM*.



# Experimental and Analytical Studies of Pteridophytes

## VIII. Further Observations on Bud Development in *Matteuccia struthiopteris*, *Onoclea sensibilis*, and Species of *Dryopteris*

BY

C. W. WARDLAW

(Department of Cryptogamic Botany, University of Manchester)

With Plate IV and twenty-one Figures in the Text

IN *Matteuccia struthiopteris* and *Onoclea sensibilis* the removal or destruction of the rhizome apex is attended by bud development at specific positions, the latter being occupied by detached meristems, i.e. superficial areas of meristematic cells which at an earlier stage formed part of the apical meristem (Wardlaw, 1943, 1943a). Buds which thus arise on older regions of the rhizome have no vascular connexion with the shoot stele. Two further observations have been made: (i) if a young bud of *Onoclea sensibilis* is excised, new buds may arise from the cavity so produced (Wardlaw, 1943a); and (ii) if a detached meristem is removed as a thin tangential section from the rhizome of *Matteuccia struthiopteris*, bud development can take place at the cut parenchymatous surface immediately below, i.e. the cortical parenchyma is potentially meristematic (Wardlaw, 1944). So far as the writer is aware, bud regeneration in ferns from cut parenchymatous surfaces has not hitherto been recorded. A somewhat similar phenomenon has, however, been observed in *Lycopodium selago* (Williams, 1933). In the Onocleoid ferns under consideration regenerative outgrowths from parenchymatous tissue have not been observed except at the specific positions indicated.

One of the aims of botanical science is to give a coherent account of the processes whereby the individual organism acquires its distinctive appearance and specific character. This involves a study of the shoot apex. In practice, buds and regenerative outgrowths may prove more convenient than the main apex for analytical studies of meristematic activity; the ferns under consideration afford favourable materials for this purpose. The eventual aim of such physiological investigations as may be undertaken will be to give an account of the development of organs and tissues in terms of the process of growth: as an essential preliminary the morphological and histological facts should be clearly ascertained. Relevant observations are given in the present paper.

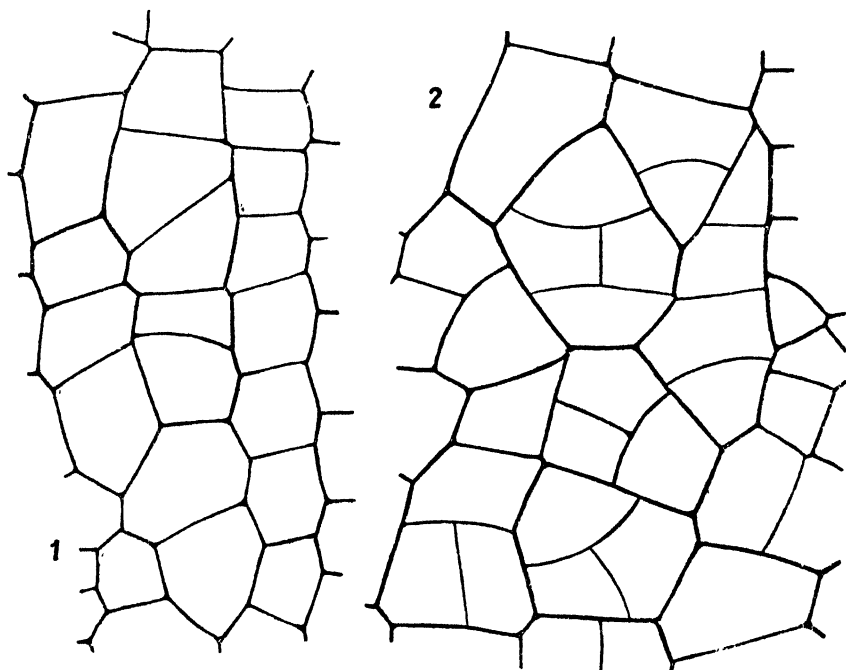
### DEVELOPMENT OF BUDS IN MATTEUCCIA AND ONOCLEA

If rhizomes of *M. struthiopteris* and *O. sensibilis* are decapitated and placed in moist peat in a cool greenhouse or in a germinator at 22°–25° C., the

development of buds takes place in the course of a few weeks. In the normal rhizome the detached meristems from which buds arise are in a quiescent condition.

*Matteuccia struthiopteris*

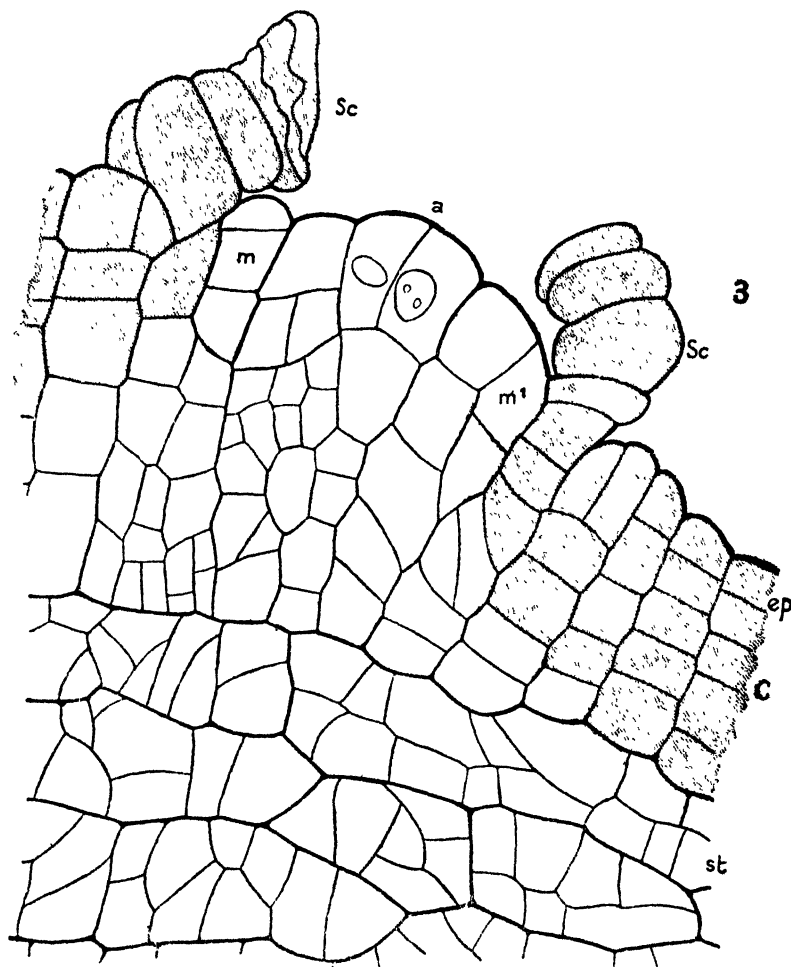
In this fern the detached meristem consists of an elongated area of superficial, prism-shaped, meristematic cells lying in a groove. When growth is



TEXT-FIGS. 1-2. *Matteuccia struthiopteris*. Fig. 1, downward view of a portion of a detached meristem on the renewal of growth; the meristematic cells are dividing by transverse walls. Fig. 2 shows other division patterns which may also be observed. ( $\times 340$ .)

renewed in a detached meristem, the superficial cells begin to divide by anticlinal walls, chiefly in the transverse plane, and to bulge outwards, Text-figs. 1 and 2. Tangential divisions follow. At the same time the outermost cortical cells also begin to divide, and this process gradually extends inwards. On further growth a hemispherical mass of pale green tissue begins to protrude from the surface of the rhizome. This outgrowth now elongates, its distal end being occupied by a cap of meristematic cells. An apical cell becomes apparent in the centre of the distal meristem (Text-fig. 3), which soon thereafter gives rise to a first and then to a second fleshy scale-leaf. These are followed by the formation of small adult leaves. Root development also takes place at an early stage. In brief, the bud rudiment rapidly gives rise to a small leafy shoot.

In rhizomes which had been kept in a cool greenhouse one large outgrowth was usually observed at each detached meristem, but not all the meristematic cells were involved. In rhizomes which had been placed in the germinator (at a higher temperature and humidity than the greenhouse) several buds,



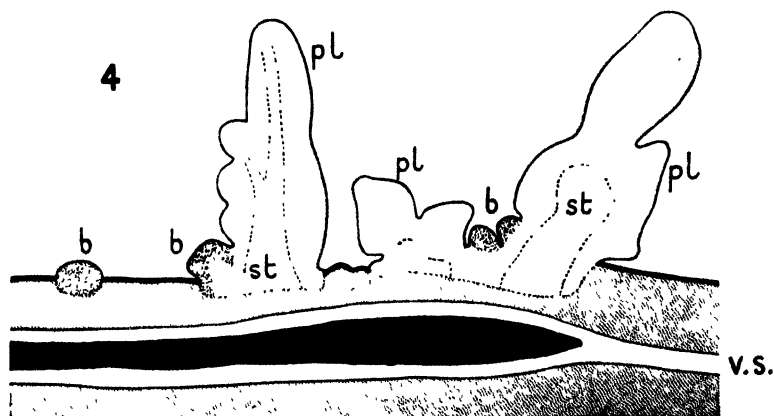
TEXT-fig. 3. Longitudinal section through a bud which is in the course of differentiating an apical cell (*a*); *sc*, scale; *m-m'*, apical meristem; *st*, vascular tissue, unshaded, showing the characteristic division of some of the larger cells; *ep*, epidermis; *c*, cortex (stippled). ( $\times 340$ .)

forming a linear group, developed at each detached meristem (Text-fig. 4). The number of buds in any one group may be quite considerable, but only two or three develop into shoots.

Some of the incipient buds, i.e. masses of meristematic tissue which stain deeply with Delafield's haematoxylin, quickly lose their meristematic potentiality and develop into hemispherical parenchymatous bodies, described as

'coralloid growths'. In other instances the developing meristematic tissue becomes parenchymatous in one region but remains meristematic in another, the enlargement of the former being the more rapid. Behind the region which remains meristematic the initial differentiation of vascular tissue can invariably be observed (Wardlaw, 1944). This tissue may be distributed in a fan-shaped pattern, the focal point being the superficial meristem (Pl. IV, Fig. 1).

As indicated above, the bud meristem for some time consists of a superficial layer of densely protoplasmic, actively dividing cells in which no single apical initial can be observed; at an early stage the marginal limits of such a meristem



TEXT-FIG. 4. *Matteuccia struthiopteris*, longitudinal radial section of a rhizome, traversing a detached meristem from which a linear group of buds has developed. Some of the buds have developed into plantlings (*pl*) each with a central stele (*st*), but other buds (*b*) (dark stippling) have remained as deeply staining masses of meristematic tissue; *v.s.* vascular strand of parental rhizome; xylem in solid black. ( $\times 14$ .)

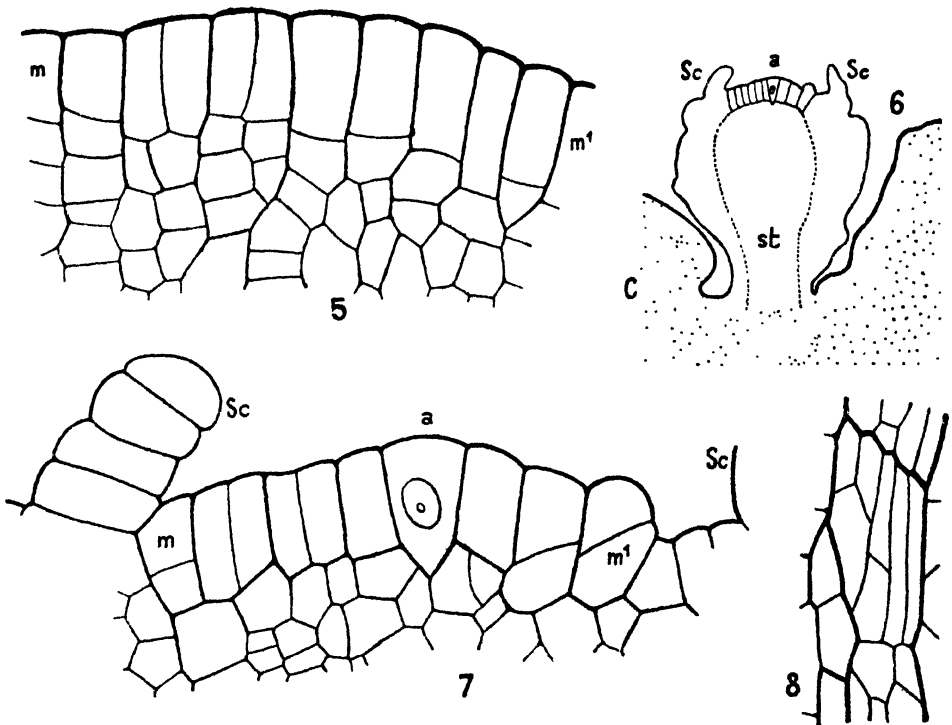
are defined by the development of scales. Sooner or later a single centrally placed cell enlarges and can be recognized as the apical cell (Text-fig. 3); thereafter further growth is attended by the production of leaves and roots. Round the base of such a plantling small tissue protuberances may be irregularly distributed: some of these, which consist of meristematic cells arranged in palisade-like rows, are capable of developing into plantlings should the initial plantling be destroyed; others which have lost their meristematic potentiality consist of large-celled coralloid growths.

### *Onoclea sensibilis*

Bud development in this fern is in general similar to that described above. Where a single bud develops, the meristematic tissue is carried upwards on the distal end of the short shoot, and considerable growth may take place before a single apical cell becomes differentiated. Text-fig. 5 shows an early stage of growth in a detached meristem, and Text-figs. 6 and 7 a bud in the

distal meristem of which the apical cell has just appeared. At this stage the deeply staining vascular tissue can be seen extending backwards from the apical meristem. Text-fig. 8 indicates the characteristic appearance of a cell during division into the smaller elongated units typical of incipient vascular tissue.

If this apical region of a bud such as that shown in Text-fig. 6 or of an older bud is damaged, a remarkable growth development becomes evident in the

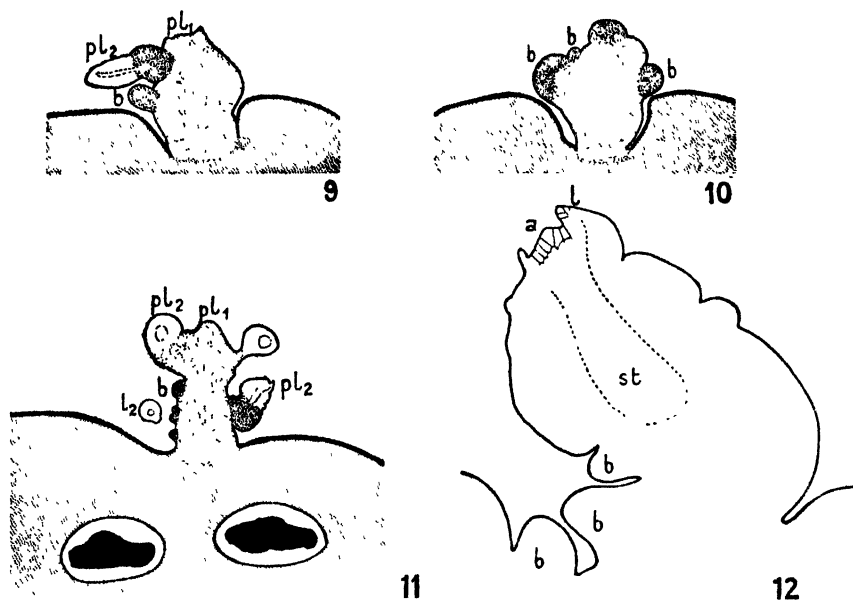


TEXT-FIGS. 5-8. *Onoclea sensibilis*. Fig. 5. Cells of a detached meristem, as seen in a transverse section of the rhizome, beginning to divide by longitudinal radial and tangential walls. Fig. 6. A small bud which has developed from a detached meristem; in the distal meristem an apical cell, shown in detail in Fig. 7, has just been differentiated. Fig. 8. Characteristic appearance of dividing cells of the stele. *m-m'*, meristematic cells; *sc*, scale; *st*, stele; *a*, apical cell; *c*, cortex of parent rhizome. (Figs. 5, 7, 8,  $\times 340$ ; Fig. 6,  $\times 60$ .)

basal region of the initial bud. This region evidently remains potentially meristematic and, as soon as the dominance of the plantling meristem is removed, active development begins at a number of irregularly placed growth centres. Two specimens showing such a development have been illustrated in an earlier paper (Wardlaw, 1943*b*). These have now been examined by means of serial sections. Several types of growth development have been observed. Firstly, there is the formation of many small hemispherical masses of darkly staining meristematic tissue, dividing chiefly by anticlinal walls and



so forming palisade-like rows of cells. Some of these tissue masses continue to grow, differentiate an apical cell, and form vasculated plantlings (Text-figs. 9–11); a plantling thus typically appears as an outgrowth of one of the deeply staining meristematic masses (Text-figs. 11 and 12). Many of the small meristematic masses do not develop further and are to be seen, sometimes singly, sometimes in considerable numbers, near the base of newly developed plantlings, i.e. those of the second order. Secondly, many of the meristematic masses may lose their meristematic character and develop into large-celled, highly vacuolated, parenchymatous masses, without vascular tissue; in fact,



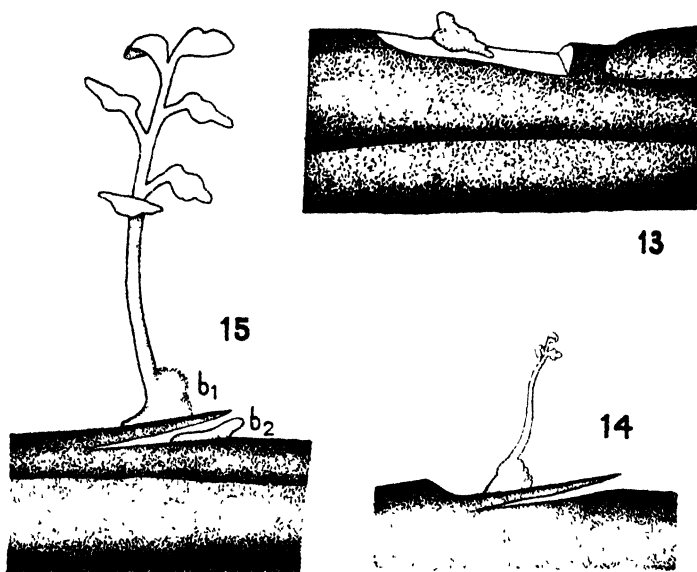
TEXT-FIGS. 9–12. *Onoclea sensibilis*. Figs. 9 and 10. Two serial sections of the same material showing meristematic activity at the base of an induced plantling ( $pl_1$ ) of which the apex has been destroyed. Many buds ( $b$ ) consisting of meristematic tissue and a plantling of the second order ( $pl_2$ ) are shown. Fig. 11. A second, injured induced plantling ( $pl_1$ ), at the base of which many buds ( $b$ ) and some plantlings of the second order ( $pl_2$ ) have appeared. The plantling on the right-hand side (below) which has grown out of a deeply staining meristematic mass is shown in greater detail in Fig. 12;  $a$ , apical cell;  $l$ , first leaf;  $st$ , stele. Cortex of parent rhizome (close stippling). (Figs. 9–11,  $\times 11$ ; Fig. 12,  $\times 70$ .)

into typical coralloid growths. Here and there, among the coralloid growths, meristematic masses in their original state may be observed; these are easily picked out by reason of their small deeply staining cells. Instances have also been observed of meristematic masses which had developed to the point of differentiating an apical cell and had been then transformed into a structure consisting of large-celled parenchyma. Such observations give some idea of the different growth developments of which detached meristems are capable.

REGENERATION FROM CORTICAL PARENCHYMA IN *MATTEUCCIA*  
*STRUTHIOPTERIS*

The observations set out in this section show that meristematic properties similar to those shown by the superficial cells of a detached meristem are shared by the cells of the cortical parenchyma which lie immediately below.

Pieces of decapitated rhizome from which the detached meristems had been removed by paring off thin slices of superficial tissue were placed on peat and



TEXT-FIGS. 13-15. *Matteuccia struthiopteris*. Fig. 13. Bud developing on the cut parenchymatous surface of a rhizome. Fig. 14. A plantling which has developed on a semi-isolated slip of tissue containing a detached meristem. Fig. 15. As in Fig. 14, but a second bud,  $b_2$ , has developed on the cut parenchymatous surface. ( $\times 3.5$ .)

maintained under the conditions already indicated. After a few weeks small outgrowths, recognizable as plantlings, developed on the cut surface of some of the specimens (Text-fig. 13). Control specimens, in which superficial layers were removed from regions other than those occupied by detached meristems, yielded no new growths.

Serial sections show that the small plantling in Text-fig. 13 originated as an outgrowth of parenchymatous cells at the cut surface (Pl. IV, Fig. 2). The relative position of the bud to the parent shoot is precisely the same as that of a plantling which has developed from a detached meristem, i.e. it has originated in a region of meristele conjunction (Wardlaw, 1943, 1943a). In other words, the regenerative outgrowth has arisen from parenchymatous tissue immediately underlying the detached meristem. Although the base of

the bud lies in close proximity to the meristele (Pl. IV, Fig. 2), it has no vascular connexion with it. There is evidence, however, that the tissues of the meristele have been affected by the development of the bud.

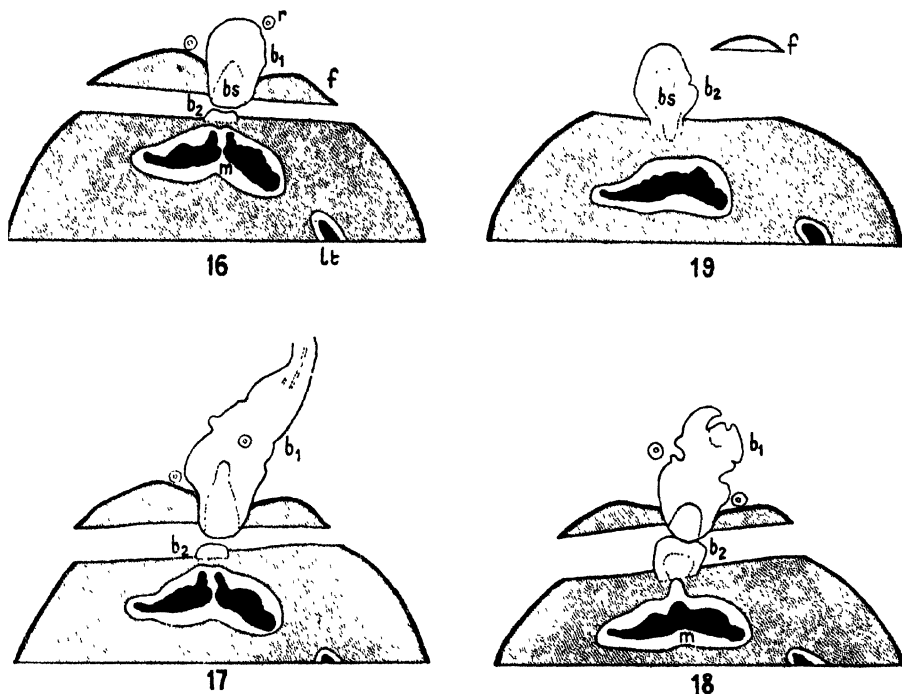
In the region where the bud stele abuts on the inner cortical parenchyma of the parental shoot, the cells of the latter have been considerably modified, their dense, deeply staining contents being in marked contrast to the adjacent parenchyma not so affected. The meristele tissues in proximity have also been affected, the cells of the endodermis and pericycle being considerably enlarged and containing dense protoplasmic contents (Pl. IV, Fig. 3). As a result, the endodermis tends to bulge outwards and in some sections its identity is almost lost (Pl. IV, Figs. 5, 6, 7). Although changes of this kind may be observed, no phloem-to-phloem or xylem-to-xylem connexion between bud stele and meristele was established in the specimen under consideration. That activating substances diffusing from the growing bud backwards into the meristele were responsible for these histological changes seems a feasible hypothesis (Wardlaw, 1944): alternatively, the histological changes observed may be related to the fact that the modified cells lie in the path of nutrients moving from the meristele to the growing bud. The cells of the phloem and xylem parenchyma were also affected, the enlargement of the latter causing some displacement of the tracheides (Pl. IV, Fig. 3). These several points are readily apparent when the differential staining of the affected meristele is compared with that of an unaffected one at the other side of the rhizome. The xylem, too, shows evidence of modification, a yellowish colour replacing the normal bright red safranin staining.

In another series of experiments with decapitated rhizomes detached meristems were separated from the rest of the shoot, except for a narrow isthmus of tissue, as shown in Text-fig. 14. The plant which has grown out originated from a detached meristem. Immediately below the base of this plantling, groups of parenchymatous cells at the cut surface of the rhizome were found to be in a state of division by tangential and radial walls, the staining reactions being those which are characteristic of developing bud tissue. It is conceivable that this development may have taken place in response to substances diffusing from the base of the bud into the film of water which at certain periods would be present between the superficial slip of tissue and the exposed rhizome tissue. Since regenerative growth can take place at the cut parenchymatous surface, as illustrated in Text-fig. 13 and Pl. IV, Fig. 2, the alternative explanation that certain regions of cortical parenchyma are potentially meristematic is probably the correct one.

In the specimen illustrated in Text-fig. 15, buds have developed both on the semi-isolated flap ( $b_1$ ) and at the cut surface of the rhizome below ( $b_2$ ); i.e. a combination of the conditions shown in Text-figs. 13 and 14 has been obtained. Text-figs. 16–19 are based on representative transverse sections of the rhizome illustrated in Text-fig. 15, from the proximal to the distal end through the bud region. The bud on the semi-isolated flap ( $b_1$ ) has arisen in the stem groove by the development of a detached meristem, its position, as

is usual, being in proximity to a point of meristele conjunction (Text-figs. 16 and 17). The median plane of the other bud ( $b_2$ ) lies almost immediately below that of ( $b_1$ ) (Pl. IV, Fig. 4).

The outer bud or plantling ( $b_1$ ) in Text-fig. 15, shows the histological and morphological features usually observed in induced plantlings. As a result of growth extension the base of the plantling bulges downwards and abuts on



TEXT-FIGS. 16-19. *Matteuccia struthiopteris*. Serial transverse sections in acropetal succession of the specimen illustrated in Text-fig. 15, showing the outer bud ( $b_1$ ) which has developed from the detached meristem and the inner bud ( $b_2$ ) which has arisen from the cut parenchymatous surface.  $f$ , semi-isolated superficial flap which contained the detached meristem;  $m$ , shoot meristele (two meristeles at point of conjunction);  $lt$ , leaf-trace;  $bs$ , bud stele. In Fig. 18 the bulge in the endodermis of the meristele in relation to the development of the inner bud ( $b_2$ ) is clearly shown. ( $\times 11$ .)

the outer tissues of the inner bud ( $b_2$ ). The position of the vascular tissue is indicated in Text-figs. 16-18; phloem, xylem, and vascular parenchyma can be recognized, these tissues extending almost to the base of the plant. The latter region consists of elongated cells divided by many transverse walls and suberized on the free lower surface. The tracheides in the lower region are typically short and sometimes of irregular shape.

The first stage in the development of plantlings at the cut surface consists in the division of uninjured cortical cells situated on or below the surface, the latter being suberized and of a deep brown colour. The initial divisions are usually by walls lying parallel to the cut surface, then at right angles to it, but

divisions in other planes are also common. At first divisions only occur in isolated cells (Pl. IV, Fig. 8), but soon a majority of the cells in the potentially meristematic region become involved and a mass of tissue actively dividing by tangential walls and staining deeply with haematoxylin begins to rise from the cut surface (Pl. IV, Fig. 9). This mass of tissue continues to grow in this manner for some time, but sooner or later a distal meristem with an apical cell becomes organized and thereafter a typical plantling with axis and leaves can be recognized.

Serial sections through the inner bud ( $b_2$ ) in Text-fig. 15, and others which have been examined, afford evidence of the influence which the developing bud may exercise on the tissues of the inner cortex and meristele. Contemporaneously with the development of the bud at the cut surface, more deeply seated cortical cells also begin to divide by tangential and radial walls. As this process extends inwards, cells of the endodermis and pericycle of the rhizome meristele also begin to enlarge and divide (Pl. IV, Fig. 5). Only those endodermal cells in the axis of the vascular tissue of the bud have been thus affected in the specimens so far examined, but the pericycle may show more extensive growth activity, including enlargement and division by tangential walls (Pl. IV, Fig. 6). As a result a bulge develops on the outer surface of the meristele (Text-fig. 18; Pl. IV, Fig. 7); the endodermis becomes extended and displaced and may lose its identity. As these developments within the meristele take place side by side with comparable developments in the inner cortex, and these in turn with the developing vascular system of the bud, the stele of the latter may become more or less completely conjoined with that of the shoot. This depends on the state of differentiation of the parental rhizome at the time of bud induction.

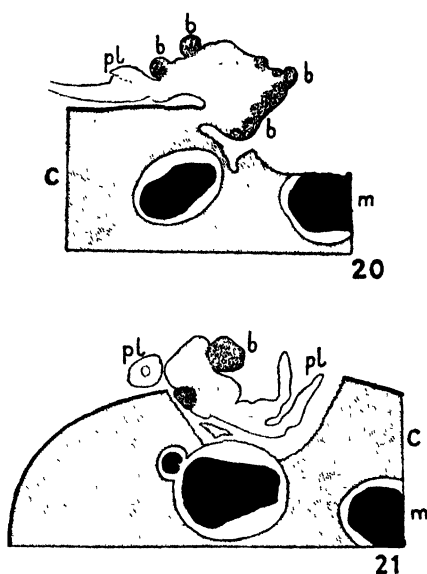
#### REGENERATION FROM CUT SURFACES IN *ONOCLEA SENSIBILIS*

In *Onoclea sensibilis* the regenerative growth obtained from cavities left by the excision of buds proceeds from the sides of the cavity (Text-figs. 20, 21). As these growths were only examined after a considerable development had taken place the present account is necessarily exclusive of the initial stages. The main mass of new tissues (Text-fig. 20) consisted of parenchyma on the surface of which numerous irregularly placed deeply staining masses of meristematic tissue were disposed. It may be assumed that all of these masses or incipient buds were potentially capable of developing into plantlings. Actually four distinct conditions were observed: (i) buds in a quiescent state; (ii) enlarging buds; (iii) buds which had become parenchymatous throughout and had developed into large-celled coralloid growths, and (iv) buds which had developed into plantlings. Buds in condition (i) were generally observed at the base of plantlings or in close proximity thereto and may be inhibited; those in condition (ii) sooner or later differentiate a terminal meristem with an apical cell and give rise to a plantling; those in condition (iii) suggest that notable differences in nutrition may occur within a comparatively small mass

of tissue. The factors that make for the development of parenchyma are, however, quite insufficiently understood.

#### RELATED OBSERVATIONS IN SPECIES OF DRYOPTERIS

In experiments with *Dryopteris* it has been shown that the proximity of an induced bud to the shoot apex determines whether its vascular system will become completely or partly conjoined with that of the shoot stele, or have no



TEXT-FIGS. 20-1. *Onoclea sensibilis*. T. S. Rhizome showing regenerative growths, consisting of parenchyma (light stippling), buds (*b*), i.e. deeply staining masses of meristematic cells and plantlings (*pl*). These regenerative growths have developed from cavities from which induced buds had been excised. *c*, cortex of rhizome; *m*, meristele. ( $\times 11$ .)

connexion with it but end blindly in the outer cortex (Wardlaw, 1943*b*). Buds have been observed of which the phloem had become confluent with the phloem of the shoot stele but where no xylem-to-xylem connexion had been effected. In other instances the tissues of the bud stele have been completely confluent with those of the shoot stele at the apical (distal) end of the region of conjunction, but separate from them lower down, i.e. the bud stele lay on the outer side of the shoot stele and gradually faded out. Now, in an experimentally induced bud, that part of the bud stele which traverses the cortex is formed from cells which were initially differentiated as cortical parenchyma. These cells undergo characteristic divisions and become transformed into vascular tissue. All the evidence is consistent with the view that this series of changes is related to the diffusion of activating substances from the apical meristem of the bud. In those instances where the bud stele occupies a position on the outside of a shoot meristele it has been observed that the pericycle

of the latter has been stimulated to growth and division. The endodermis may also be affected as in the case of *Matteuccia struthiopteris* described above.

#### HETEROAUXIN AND BUD DEVELOPMENT

In flowering plants it is now well known that if the shoot apex is removed the inhibited axillary buds will develop. If, however, the surface exposed on removing the shoot apex is smeared with 3 per cent. heteroauxin in lanoline, the axillary buds remain inhibited. Similar experiments, with appropriate controls, have now been carried out with rhizomes of *Matteuccia struthiopteris* and *Onoclea sensibilis*, and results comparable with those indicated above for flowering plants were obtained; i.e., no plantlings developed from the detached meristems in decapitated rhizomes which had been treated with heteroauxin.

#### DISCUSSION

It has already been noted (Wardlaw, 1943a) that a detached meristem is not strictly speaking a bud primordium or rudiment, in that, on development, it does not necessarily give rise to one particular organ, i.e. to a bud (in the sense of a small shoot with leaves). The evidence now presented shows that both the superficial cells of the detached meristem and the underlying cortical parenchyma are capable of meristematic activity when the shoot apex is removed. In *Matteuccia struthiopteris* and *Onoclea sensibilis* these potentially meristematic tissues are situated in the region of a conjunction of meristeles. Such positions may be characterized as being those which have been least affected by the growth expansion of the leaf bases; i.e. during the development of the rhizome they are regions of minimal mechanical stress and of minimal parenchymatous development. In other words, the less the parenchymatous development the greater is the residual meristematic potentiality. This hypothesis would also account for the position of lateral buds in species of *Dryopteris*.

The question may perhaps be raised as to whether the meristematic potentiality of the cortical parenchyma in the positions indicated is a residual property, i.e. that it is referable to the initial meristematic condition of the tissue when it was formed at the apex, or whether it is due to metabolites diffusing inwards from the meristematic cells of the detached meristem. This is a question which cannot be definitely answered at this stage, but, in view of the quiescent condition of detached meristems in the normal rhizome and of the development of buds when the superficial tissue has been removed, the evidence may perhaps be considered to support the first alternative. Once growth has been renewed in the detached meristem there is evidence that the underlying cells, including the tissues of the meristele, may be affected by the inward diffusion of activating substances, the pericycle being particularly reactive.

During the development of buds at detached meristems the following successive events are to be observed: (i) breaking of the resting or inhibited condi-

tion consequent on the removal of apical meristem; (ii) cell division in the detached meristem and the inward transmission of the growth stimulus; (iii) the development of a small hemispherical mass of meristematic tissue, i.e. of a bud, or of several buds; (iv) the development of a distal bud meristem consisting of distinctive prism-shaped meristematic cells and the internal differentiation of vascular tissue; (v) the differentiation of a more or less conspicuous apical cell and the inception of a regular system of apical segmentation; (vi) the development of leaf primordia; and (vii) the development of root primordia. These may be indicated as the phenomena which await physiological investigation.

The sequence of events culminating in the development of a plantling, as described above, is not invariably followed: it has been seen that a detached meristem may also develop in other ways. For the present purpose, however, the main interest lies in the development of the leafy-shoot type of organization. In the ferns under consideration leaf primordia are apparently not formed prior to the differentiation of the apical cell. In other words, the organization of an apical meristem, comprising an apical cell and the associated meristematic cells, is a necessary prior condition for the development of a leafy shoot. If this finding can be substantiated, it will constitute an important advance in the investigation of the apical meristem and may eventually lead to a better understanding of the fundamental problems of morphogenesis. It is well known that if the apical cell of a fern shoot is destroyed by needle-puncturing the apical meristem ceases to function, the further growth of the shoot being carried on by a lateral bud and not by an undamaged adjacent meristematic cell. Here it is appropriate to note that as the facts relating to the organization of bud meristems become more clearly understood they will have to be brought into line with those which relate to the embryogeny of the sporophyte.

Many different types of bud development in ferns have been described (Goebel, 1908; Bower, 1923; Williams, 1938), but in all of these the new growth springs from uninjured superficial cells. So far as the writer is aware regenerative growth from exposed cortical surfaces has not hitherto been recorded. The observations described here, therefore, break new ground. They show that the cortical tissue of some leptosporangiate ferns behaves like that of the Dicotyledons, in which group regenerative growth from cut surfaces is well known.

In a study of bud and root regeneration in flowering plants Priestley and Swingle (1929) came to the conclusion that the behaviour of a living meristematic cell 'is determined by its position in a group and the behaviour of the group by its relative position in a complex organization. Potentially any cell is capable of meristematic growth and may produce any cell organization characteristic of the species; practically, however, its possibilities are definitely limited by its position in a complex organization' (p. 85). Organization, in fact, is the basis of directed meristematic activity, and theories of formative substances, of qualitative or quantitative metabolic differences, of different preformed



'anlagen' (primordia) are each and all in some respect found to be inadequate. 'Relative position in a complex organism thus seems to determine whether each individual cell shall be meristematic and densely filled with protoplasm, semi-meristematic, vacuolated and still dividing, or greatly distended with sap and showing no signs of growth activity.' The fern data now available show that bud development takes place at certain specific positions (i.e. positions occupied by detached meristems) and nowhere else. In the ferns, primordia are, in fact, involved, each such primordium having originally constituted part of the apical meristem. These primordia persist in interfoliar positions which have been subject to minimal distension during growth.

In *Matteuccia struthiopteris* a detached meristem may give rise to a linear group of buds. This state of affairs in the ferns affords a parallel condition to that found in certain flowering plants. Thus Sandt (1925) has described instances in which buds ('Beiknospen') may be produced in series above, below, or on the flanks of the first buds.

Holden (1912) observed that when fern leaves are wounded in the apical region the superficial cells in the injured areas undergo divisions comparable with those illustrated here, and a protective cambiform layer is formed. The older the region of the petiole that is wounded the less is the response by cell division. Plants wounded in the basal region of the petiole generally showed no cell-elongation, but various types of wall thickening and deposition of gum were constant features. The illustrations in Pl. IV, Figs. 3, 4, indicate that in the adult rhizome of *Matteuccia struthiopteris* the cortical parenchyma shows no response to wounding, other than by superficial suberization and gum deposition, except in those regions which lie immediately below detached meristems. The exposed cortical parenchyma in these regions not only shows meristematic activity but gives rise on further growth to buds and plantlings. Holden (1916) has also shown that the vascular tissues of ferns may react to wounding by the elongation and division of the cells of the pericycle and conjunctive parenchyma. Comparable changes recorded in the present investigation have been associated with the development of buds.

#### SUMMARY

1. An account is given of the development of lateral buds in the ferns *Matteuccia struthiopteris*, *Onoclea sensibilis*, and species of *Dryopteris*, special reference being made to hitherto unrecorded regenerative outgrowths from exposed cortical tissue.

2. Buds arise at specific points on the shoot from the superficial cells of detached meristems or from the underlying cortical parenchyma, but nowhere else. The successive stages of growth leading to the development of plantlings are illustrated, described, and discussed.

3. The organization of an apical meristem, comprising an apical cell and the associated superficial meristematic cells, appears to be an essential antecedent condition for leaf development.

4. Not all detached meristems give rise to a single bud. Several buds in linear series and parenchymatous coralloid growths may also be produced under conditions which are described.

5. The investigation has afforded evidence of the influence which the developing bud may exercise on the tissues of the inner cortex and meristele of the parent rhizome. Deep-seated cortical parenchyma undergoes cell divisions and is transformed into vascular tissue; the endodermis, pericycle, and stelar parenchyma may also be stimulated to growth by substances proceeding from the bud meristem.

The writer has pleasure in acknowledging the assistance received from Mr. E. Ashby in microscope preparations and photographic illustrations.

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#### EXPLANATION OF PLATE IV

Illustrating Professor C. W. Wardlaw's article, Experimental and Analytical Studies of Pteridophytes, VIII.

The figures are from untouched photographs.

#### *Matteuccia struthiopteris*

FIG. 1. Longitudinal section of a detached meristem showing a bud meristem (left) with developing vascular tissue fanning out below and a conspicuous parenchymatous development (right). ( $\times 60$ .)

FIG. 2. Transverse section of a rhizome showing a bud arising from exposed cortical parenchyma in proximity to a region of meristele conjunction. ( $\times 25$ .)

FIG. 3. Part of a meristele underlying a developing bud. The endodermis (*e*) and the pericycle have been stimulated to growth, so that the identity of the former is almost lost. Cells of the conjunctive and xylem parenchyma have also enlarged and caused some displacement of the tracheides. ( $\times 210$ .)

FIG. 4. Transverse section of a rhizome showing a bud on the semi-isolated epidermal flap and a second bud arising from the cortical parenchyma. The buds occur in a region of meristele conjunction. ( $\times 25$ .)

FIG. 5. Transverse section of a rhizome showing part of a meristele underlying a developing bud. Cell divisions can be seen in the inner cortical parenchyma and in the endodermis (*e*). ( $\times 180$ .)

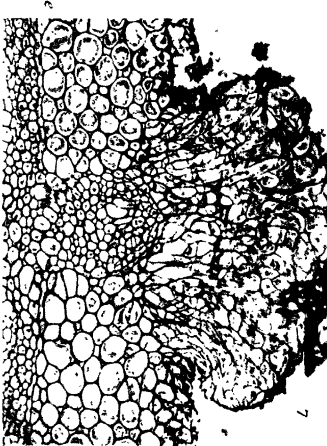
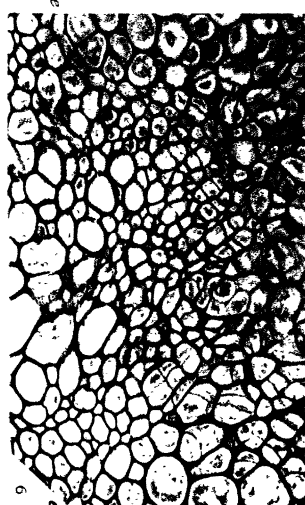
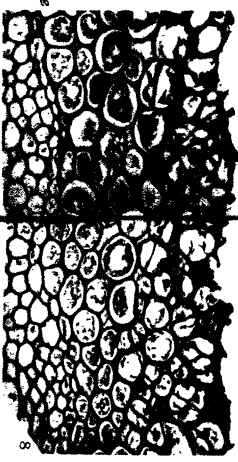
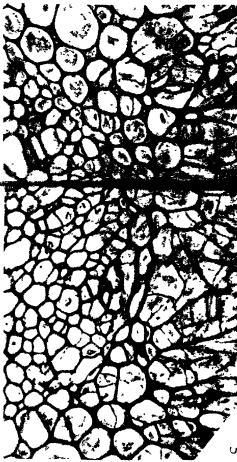
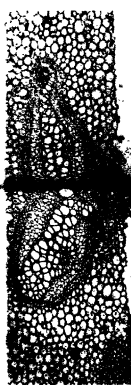
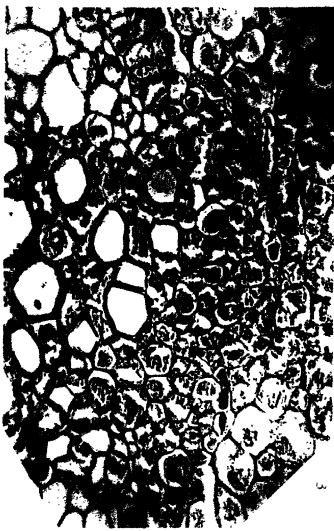
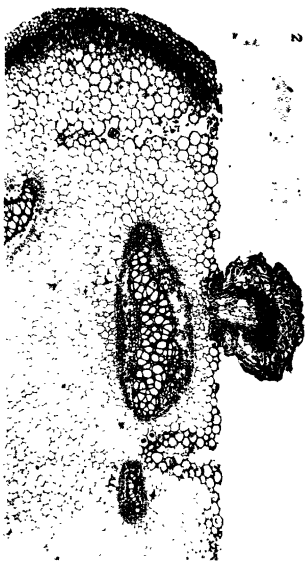
FIG. 6. Tissues of a meristele underlying a developing bud. Cell divisions can be seen in the inner cortex, and in the endodermis (*e*), which in part has lost its identity. Cells of the pericycle have undergone radial elongation and show well-marked combiform divisions. ( $\times 180$ .)

FIG. 7. Typical appearance of a young bud developing from an exposed parenchymatous surface. The rhizome meristele shows a well-marked bulge below the bud. ( $\times 85$ .)

FIG. 8. Early stages in bud development in exposed parenchymatous tissue. Divisions can be seen in the outermost parenchymatous cells. *e*, endodermis of meristele. ( $\times 210$ .)

FIG. 9. A later stage in bud development in exposed parenchymatous tissue. ( $\times 210$ .)





WARDLAW — BUDS OF MATTEUCIA STRUTHIOPTERIS.



# **The Germination of the Seed of *Striga lutea***

## **II. The Effect of Time of Treatment and of Concentration of the Host Stimulant**

BY

R. BROWN AND M. EDWARDS

*(Department of Botany, University of Manchester)*

With six Figures in the Text

### INTRODUCTION

IN the first paper of this series (Brown and Edwards, 1944) it was shown that the effect of the host stimulant necessary for germination varies with the time of exposure of the seed to a previous treatment, the 'pretreatment'. This treatment involves incubating moist seeds at a fairly high temperature for some days. It has been shown that as the pretreatment period is extended the percentage germination resulting from standard stimulation increases until a maximum value is reached, and then declines gradually to a negligible quantity.

This observation indicates that during pretreatment the state of the seed changes with time, but it does not show whether the change is independent of the concentration of the stimulant; an examination of this aspect of the position is clearly necessary for an elucidation of the nature of the changes that occur in pretreatment. In this connexion data are required on the effect of dilution of the standard solution on germination at different stages of pretreatment, and of the time of exposure of the seed to the stimulating solution, since the rate of absorption of the active substance may change and may affect the extent to which concentration limits germination. Data that meet this requirement have been obtained and are presented here, although they are part of the results of an investigation designed for another purpose, that of evolving a biological test for the stimulatory capacities of various preparations.

### EXPERIMENTAL METHODS

The variables of the investigation, concentration, and time of application of the stimulatory solution were incorporated as modifications of an experimental design which involved (1) pretreating the seed, (2) producing a standard solution of the natural stimulant, and (3) testing the effect of any given solution. The particular techniques used for these purposes were based on methods developed in another investigation and have been described elsewhere (Brown and Edwards, 1944).

The several concentrations of the stimulant were prepared by appropriate dilution of a standard solution with glass-distilled water. The time during

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which the seed was exposed to any particular concentration was varied by transferring the seed, after immersion in the stimulant, for different times, to distilled water, if the time of immersion was less than 24 hours. In all cases the counts for estimating percentage germination were made 24 hours after the beginning of the treatment with the stimulant, and therefore different periods of exposure to the stimulant involved different complementary times of immersion in distilled water.

In all the experiments of the present series seeds were pretreated by incubating cultures at 22° C. in the dark. At intervals of 7 days groups of samples were taken, and with each group the effects on germination of the standard solution, and of concentrations of 1/5, 1/10, 1/50, 1/100, 1/250, 1/500, 1/1,000 of the standard solution, were investigated; moreover, the effect of each concentration on each sampling occasion was determined, usually in relation to four periods of exposure, namely 2, 4, 6, and 24 hours. Each value given in the next section is the mean of six observations.

The data given below were all obtained with the same sample of seed, which originally came from Nyasaland; but they are the results of two separate experimental series, the second of which was conducted six months after the first. The earlier is designated below as expt. 1 and the later as expt. 2.

#### EXPERIMENTAL RESULTS

As indicated above, a series was included in each experiment in which the seeds were treated with the undiluted standard solution for 24 hours. The results obtained at each stage of pretreatment in expt. 2 are shown in Fig. 1, and they confirm the earlier published results obtained with the same treatment. As pretreatment is extended, percentage germination at first increases until a maximum is reached and then decreases, although it may be noted that the present data suggest a more rapid increase and a less abrupt decrease after the maximum is reached than do the earlier. These differences are no doubt related to the fact that although both groups of observations were made with the same sample, nevertheless the seed when used for the present series was about twelve months older.

The data of Fig. 1 are reproduced in Fig. 2, which also shows the germination induced by various dilutions of the standard solution at each stage of pretreatment, the seeds being exposed to the stimulant for 24 hours. The results of Fig. 2 are from expt. 2, and may be compared with more limited corresponding data obtained in expt. 1 shown in Fig. 3. Figs. 2 and 3 together indicate two important features. First, with seeds pretreated for the longest and shortest times (7, 14, 35, and 42 days), giving relatively low germination frequencies with the standard solution, percentage germination decreases progressively with increasing dilution; but with seed pretreated for the intermediate period, and giving the highest frequency with the standard solution, percentage germination decreases only when the concentration has been reduced below a certain critical level. Secondly the concentration necessary to induce any of the relatively low frequencies varies with the stage of pre-



treatment. Thus a percentage germination of 30 is induced by the undiluted solution when the period of pretreatment is 7 days, and by a concentration of  $1/250$  of the standard when the period is 21 days. As pretreatment is extended the tendency is for the concentration necessary for any particular frequency to decrease until the period of 21 days is reached, and then to increase with further extension.

The results of Figs. 2 and 3 were obtained with a period of exposure of 24 hours. The effects of shorter exposures are shown in Figs. 4*a-c*, 5, and 6. The data of Fig. 4*a-c*, are from expt. 2, and those of Figs. 5 and 6 from expt. 1.

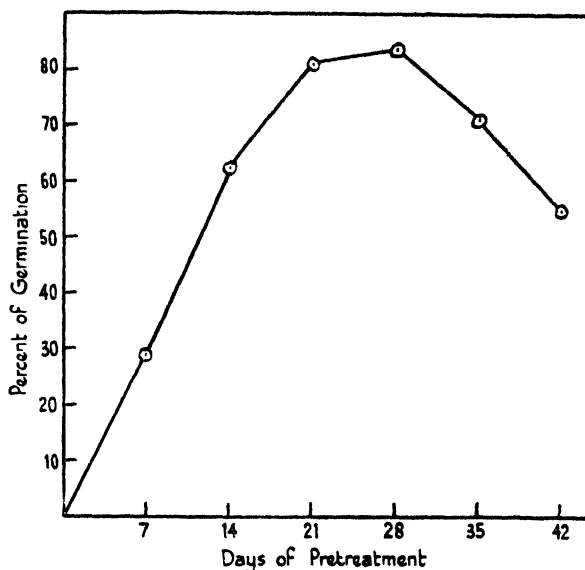


FIG. 1. Change in percentage germination with increasing time of pretreatment at 22° C. Seeds treated with standard solution for 24 hours. Data from expt 2.

In this connexion the results of the two series differ in certain important respects.

At all stages of pretreatment in expt. 2 there was no effect on prolonging the period of exposure from 2 to 4, 6, or 24 hours, and accordingly only three representative sets of data are shown in Fig. 4*a-c*. The values given with 2 hours may be slightly lower than those given with the other treatments, but the difference, if any, is negligible, and there is certainly no difference at all between the series exposed for 4, 6, and 24 hours, and that at any concentration of the stimulant. Fig. 6, on the other hand, which embodies results from expt. 1, shows that the effect of exposure for different times to the undiluted solution indicates that with seed in the state it was in when used for expt. 1 germination tends to increase at all stages of pretreatment as the period of exposure is increased from 1 hour up to at least 6 hours. Thus the two experimental series differ markedly with respect to the effect of the time of exposure to the undiluted solution, but in another connexion there is a significant

similarity. Since at all dilutions in expt. 2 there is no effect on germination of prolonging the period of exposure beyond 2 hours, it follows of course that the effect of dilution is relatively the same with all periods of exposure. In expt. 1, however, in which the period of exposure does have an effect, nevertheless the effect of dilution is still relatively always the same, whatever

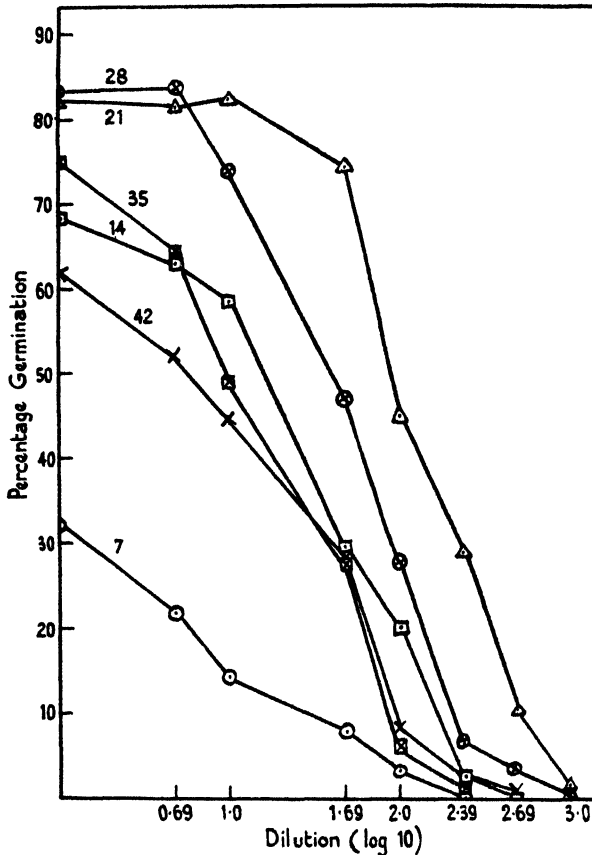


FIG. 2. Effect of dilution (1/5th to 1/1000th) of standard solution on germination of seed pretreated for different periods at 22° C. Figures attached to curves indicate days of pretreatment. The dilutions are expressed logarithmically. Data from expt. 2.

the period of exposure. The curves of Fig. 5, it may be noted, all follow the same course. It is particularly significant that the curves for the 6- and 24-hour exposures all fall as steeply, and from the same point, as the curve for the 2-hour exposure, indicating that the effect of dilution does not become relatively greater as the period of exposure is curtailed.

#### DISCUSSION OF RESULTS

It is evident from Figs. 2 and 3, since percentage germination tends to decrease with decreasing concentration (at least with seed in the earliest and

latest phases of pretreatment), that within certain limits germination is quantitatively related to the amount of the stimulant absorbed from the solution. In which case the failure of the seed to react to periods of exposure longer than 2 hours with an increased germination in expt. 1 (Fig. 4*a-c*) may have been due to an extensive reduction in the concentration of the stimulant either by breakdown or by a rapid absorption of the active principle. The standard

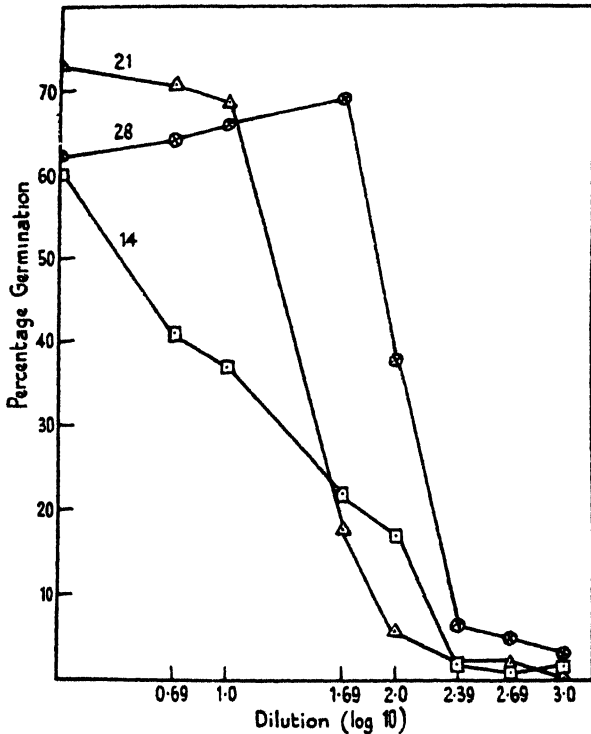


FIG. 3. Effect of dilution (1/5th to 1/1000th) of standard solution on germination of seed pretreated for different periods at 22° C. Figures attached to curves indicate days of pretreatment. The dilutions are expressed logarithmically. Data from expt. 1.

solution was employed in expt. 2 in exactly the same way as in expt. 1, in which it is evident, from Fig. 6, that it retained full activating power for at least 6 hours; the results of Fig. 4 were therefore probably not affected by a loss of activity due to decomposition of the stimulating substance. The second possibility is equally untenable. The experimental technique involved a standard volume of stimulant solution applied to a variable number of seeds, and in these circumstances, if the solution is being exhausted by a rapid absorption of the stimulating substance, then germination should decrease as the number of seeds increases. The results obtained with each concentration have been grouped according to the total number of seeds they contained, and the average germination frequencies for the different groups compared. At

all stages of pretreatment and with all concentrations the result is the same—seed number has no effect on germination frequency.

Evidently the results of Fig. 4*a-c* showing the effect of time of exposure are due to conditions of the seed and not of the solution. The evidence suggests that absorption is restricted to the first 2 hours of immersion in the stimulating solution, that this absorption is proportional to the concentration, and that even when the concentration is low the initial absorption leads to changes

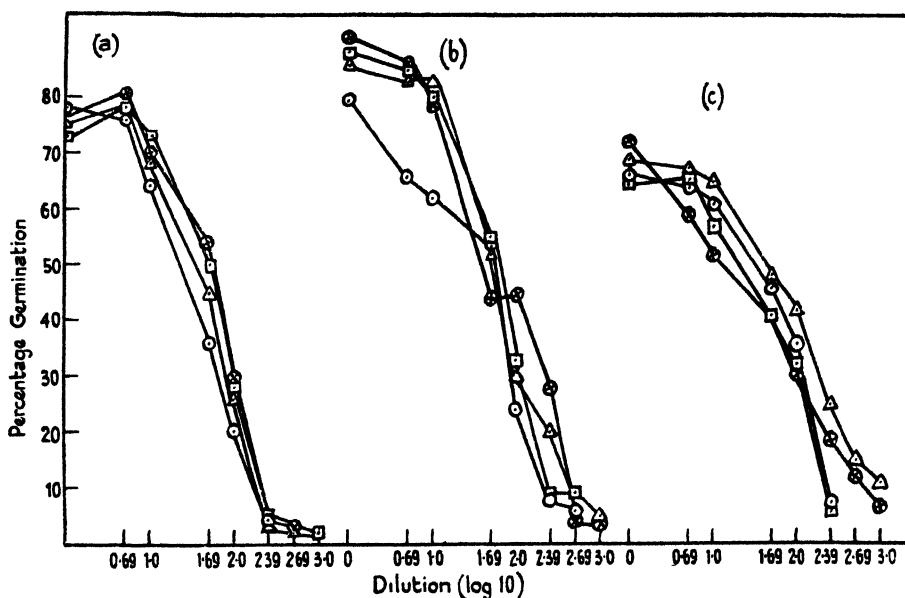


FIG. 4. Effect of time of exposure to stimulant and dilution ( $1/5$ th to  $1/1000$ th) of standard solution on germination of seeds pretreated for (a) 21 days, (b) 28 days, (c) 35 days. Each curve represents a series with the same exposure time,  $\circ$  indicates 2 hrs.,  $\square$  4 hrs.,  $\triangle$  6 hrs., and  $\otimes$  24 hrs. Data from expt. 2.

which prevent the further absorption that might be expected from a solution in which the active principle is still present. The same conclusion is suggested by the data of Fig. 5 which belong to expt. 1. In this case there is an increase in germination as the period of exposure is extended from 2 hours to 6—a fact which is emphasized by the data of Fig. 6. But it is clear from Fig. 5 that this is not due to the absorption of the stimulant increasing with the time of exposure, since this should result in percentage germination decreasing relatively more slowly at the longer exposures than at the shorter.

It is suggested that the process shows two phases, an initial phase of superficial absorption, followed by assimilation into the seedling. Now, if the interval between the two stages is comparatively long and if the substance absorbed is only held loosely on the surface on to which it is absorbed, then when the seed is transferred from the solution into water a fresh equilibrium state will tend to be established by the diffusion of some of the solute back

into the surrounding fluid. In which case, with absorption restricted to the first 2 hours during which the seed is exposed to the stimulant solution, germination will tend to decrease as the length of the subsequent period of immersion in water is increased. The longest of such periods of immersion in water are those in which the exposure to the stimulant is shortest and hence the lowest germination is with stimulation for 2 hours. Not only so, but when germination is determined by a variable period of immersion

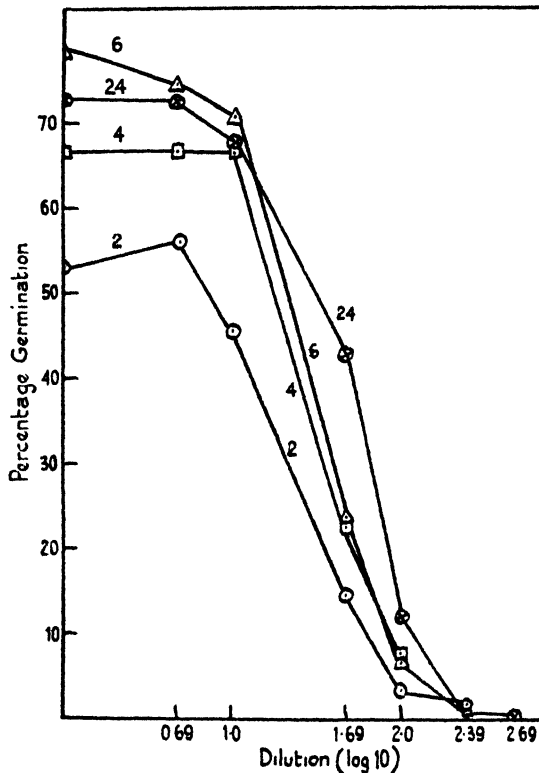


FIG. 5. Effect of time of exposure to stimulant and of dilution ( $1/5$ th to  $1/500$ th) of standard solution on germination of seed pretreated for 21 days. Figures attached to curves indicate exposure times in hours. Data from expt. 1.

with a standard period of absorption before assimilation occurs, then the change with dilution must be relatively the same whatever the period of exposure to the stimulant—which is precisely the situation indicated by the data of Fig. 5.

The conclusion of the last paragraph is of course only justified if germination at all stages of pretreatment is determined by the amount absorbed or by the amount retained after absorption. In general it is clear from the data of Fig. 2 that such is indeed the case when the seed is in the earliest and latest stages of pretreatment, and when the concentration of the solution is clearly limiting. It may be noticed, however, that at the intermediate stage of pretreatment,

when germination with the standard solution reaches a peak value, a five-fold, and in certain instances a hundred-fold, dilution of the standard has little or no effect on germination (Figs. 2 and 3). A similar effect is shown by the data of Fig. 5 in which a five-fold dilution has no effect on germination. Now this may be due to one of two conditions. Either more of the stimulant is absorbed at the higher concentration than at the lower, and some factor other than the amount absorbed is limiting; or the same amount is in fact absorbed at the two concentrations. It is significant that a five-fold dilution does not affect germination with any exposure time. In this connexion two possibilities

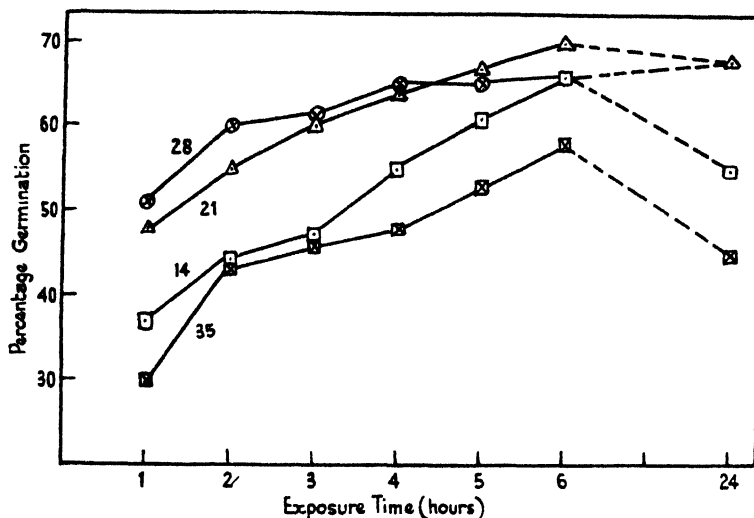


FIG. 6. Effect of different periods of exposure to the stimulant on germination of seed pretreated for different periods. Figures attached to curves indicate days of pretreatment. Data from expt. 1.

may be considered. First, the lower germination with the standard solution with 2 hours' exposure may be due to a smaller absorption of the stimulant, but if this is the case, there should be a decrease in germination when the concentration is reduced to one-fifth of the original. Secondly, absorption is complete within the first 2 hours, but different amounts are absorbed at the two concentrations, in that event there should still be a decrease in germination with an exposure of 2 hours when the concentration of the solution is reduced to one-fifth, since the lower germination with the shorter exposure to the standard indicates that the amount assimilated is the limiting factor. On the other hand, if the same amount is absorbed at both concentrations and the amount assimilated is determined by the period of immersion in water, then the condition observed would arise—that at all periods of exposure there is no decrease in germination with dilution.

Thus the data from the two experimental series both indicated that absorption is complete within 2 hours. On the other hand, while the data from expt. 1 (Fig. 5) show that the subsequent period of immersion in water affects

germination, those from expt. 2 (Fig. 4a-c) suggest that this factor has no effect. The difference undoubtedly indicates that as the seed ages the rate of assimilation after absorption increases.

Since absorption is complete within 2 hours at all the stages of pretreatment examined, the results of Figs. 1 and 2 cannot be attributed to changes in the rate of absorption of the stimulant. Nor can they be due to changes which are independent of the reaction to the stimulating substance. As indicated above, the same germination may be given by different concentrations of the stimulant at different stages of pretreatment. Before and after the peak phase of pretreatment is reached, the necessary concentration for any given germination tends to decrease and to increase respectively with time. Moreover, in the stages of pretreatment when the concentration for any given germination is relatively high, germination decreases progressively with increasing dilution, suggesting that in these stages concentration is limiting and that, with higher concentrations than that of the standard, higher percentage germination would be obtained. The data suggest that throughout pretreatment (at least between 7 days and 42 in the conditions of the present series of experiments), the peak germination given at 21-28 days could be obtained with considerably higher concentrations than that of the standard solution. At the peak stage the concentration of the standard solution is not limiting, and it can be reduced considerably before a critical concentration is reached. A similar but different critical concentration would no doubt operate at each stage of pretreatment; the critical concentration necessary for the induction of the maximum germination frequency would decrease progressively until the peak phase of 21 days is reached, and then increase with further extension of pretreatment.

The conclusion of the last paragraph is reached on the basis of observations made at intervals of 7 days, and it must be emphasized that it is relevant only to the stages of pretreatment that succeed the 7-day stage. It cannot be applied to the first 3 or 4 days, when apparently no germination can be induced with any concentration of the stimulant (unpublished data).

The change with time in the concentration necessary to induce the maximum germination suggests a tentative hypothesis as to the nature of at least one of the changes induced by pretreatment. It suggests that a certain definite quantity of the stimulant must be present in the seed before germination can occur and that the seed can itself provide part of this amount. During the early phase of pretreatment, before the peak stage is reached, the stimulating substance is formed by the metabolism of the seedling and continues to accumulate. As it accumulates a progressively smaller amount need be provided from an external source to bring the quantity in the seed to the level necessary for germination. When the peak phase has been passed, the amount in the seed is progressively reduced by changing metabolic conditions, and as a result a progressively greater quantity of the stimulant must be provided externally to induce the maximum germination. One piece of evidence has already been published which is consistent with this interpretation. It has been shown that even in cultures in distilled water a small number of

seeds always germinate. The highest proportion of the seeds that germinate spontaneously, however, occurs at the peak phase of pretreatment, when the critical concentration of the stimulant necessary for maximum germination is probably lowest. And it may therefore be suggested that this high spontaneous germination is the result of the formation by the seed of the same stimulant substance that originates with the host root.

#### SUMMARY

✓1. Data are presented which show the effect on germination of diluting a standard solution of the host stimulant, and of varying the time during which the seed is exposed to the solution.

2. The experimental design involved incubating moist seeds at 22° C. in the dark for varying periods (the pretreatment) before exposing them to the stimulant, and it is shown that with the standard solution the germination frequency increases as the period is extended to 21 days and decreases with further extension.

3. During the phases when as a result of previous treatment the germination frequency is increasing and decreasing with the standard solution, progressive dilution of this solution leads to a progressive decrease in germination, indicating that for seed in these phases the concentration of the standard is limiting, and that higher concentrations than that of the standard would probably yield higher germination frequencies. At the stage when germination is highest with the standard solution the concentration can be reduced considerably without affecting germination.

4. It is shown that with exposure to the solution for 2, 4, 6, and 24 hours the effect of dilution is, relatively, always the same, and that only in certain cases is there an increase in germination as the period of exposure is increased. These observations are taken to indicate that absorption by the seed of the stimulant from the solution is complete within 2 hours.

5. It is suggested that the effect of incubating moist seed before applying the stimulant is to decrease the concentration necessary to induce maximum germination as the treatment is extended to 21 days, and to increase it as the treatment is further prolonged. The tentative hypothesis is put forward that the seed itself forms a stimulating substance, which is the same or similar to that which originates in the host root, and that the decreasing concentration of host substance necessary to induce maximum germination during the first 21 days is due to the accumulation of the stimulating substance self-formed in the seed. Similarly that the increasing concentration necessary after 21 days is due to the metabolic decrease of the accumulated quantity.

#### LITERATURE CITED

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# Studies on Foliar Hydration in the Cotton Plant

## VII. The Size Factor<sup>1</sup>

BY

T. G. MASON

(Cotton Research Station, Trinidad, B.W.I.)

With three Figures in the Text

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### I. INTRODUCTION

IN our first paper on the *size factor* we (Mason and Phillis, 1942) described experiments in which changes in potassium supply to the roots were sometimes positively and sometimes negatively related to *hydration*.<sup>2</sup> It was found that where increased potassium supply did not lead to marked increases in *size*, the effect of potassium was to increase *hydration*, but that where large increases occurred it might decrease *hydration*. Environmental aridity appeared to be the controlling factor. When humidity was high, an increase in *size* was accompanied by an increase in *hydration*; when it was low, increased water strain, it was suggested, resulting from increased *size* might outweigh the potassium effect. It was pointed out that the work of Gregory and Richards (1929) who found that potassium supply and *succulence* were negatively correlated, and of Richards and Shih (1940, 1940a) who found no consistent relationship between potassium and *succulence*, might have been vitiated by their failure to take account of the *size factor*.

In a second paper on the size factor we (Phillis and Mason, 1943) showed that changes in potassium supply were accompanied by changes in the electrical conductivity of the sap. Potassium appeared to affect hydration as a result of its effect on the salt content of the sap. Salt content and hydration were positively correlated, but this relationship might be obscured by changes in water strain resulting from changes in size.

In a recent paper Richards (1944) has suggested that we may have over-emphasized the importance of the size factor and that other factors have not

<sup>1</sup> Paper No. 38 from the Physiological Department of the Cotton Research Station, Trinidad, B.W.I.

<sup>2</sup> For the sense in which we use the term *hydration*, see Mason and Phillis (1943).

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received adequate consideration. As many interesting and important issues are raised by Richards's paper, it is proposed to consider his criticisms at some length.

## II. THE EXPERIMENT OF RICHARDS AND SHIH

Richards and Shih conducted an experiment in which barley was grown under twenty-two combinations of nutrients. The data were submitted to statistical analysis, as a result of which the predominant effect on succulence was attributed to sodium. Potassium was the only element of those whose effects were measured which showed no appreciable relationship to succulence. The result is surprising in view of the large variation in the potassium content of the tissues.

They ascribe the apparent independence of potassium and succulence to differences in carbohydrate level.<sup>1</sup> Our suggestion that the size factor, rather than the carbohydrate level, may have been responsible is rejected by Richards. His reasons for rejecting our suggestion are as follows.

He stresses the fact that the pots we used were much smaller than those employed by Richards and Shih and that our plants (1 per pot)<sup>2</sup> were much heavier than the total crop (3 per pot) of Richards and Shih. He also emphasizes that our experiments were carried out in the tropics while Richards and Shih's were done in a temperate climate. He overlooks the fact that the period of daylight is much longer in the summer in temperate England than in tropical Trinidad and that the size factor is very liable to operate in sand-culture irrespective of size of pot. Moreover, he does not appear to appreciate that in a transpiring plant there is always some water strain. The only exception occurs when root pressure is active. What is, however, of greater importance is that he also overlooks the fact that the high-sodium nutrient solutions employed by Richards and Shih may have been toxic to the roots. Such solutions may be physiologically arid, and differences in water strain may well have prevailed between plants of different size.

He next raises what he described as a *crucial point*. 'The crucial point', he says, 'is that these small plants showed *either considerably increased or considerably decreased* leaf succulence at one-ninth the normal potassium level, according as the calcium sodium ratio was low or high.' He points out that the *relative* reduction in dry weight due to potassium starvation was similar in both the high-sodium and high-calcium series. Richards makes no attempt to explain why an increase in potassium leads to a decrease in succulence in the high-sodium series and an increase in the high-calcium series. His argument apparently is that the size factor cannot have been operative and that these different responses in succulence must consequently in some unexplained way have been due to different mineral or carbohydrate levels in the two series.

<sup>1</sup> Carbohydrate level and cell-wall extensibility are assumed to be negatively correlated.

<sup>2</sup> He is not correct in stating that our plants weighed well over 300 gm. This was the weight of 10 plants.

As we have already indicated, Richards overlooked the fact that the high-sodium solutions may have been physiologically arid. There was very little calcium in the high-sodium solutions and such unbalanced solutions are in our experience toxic to roots. Both Russel (1938) and Richards and Shih comment on the toxic effects of these high-sodium solutions. The size factor may well have assumed importance in such solutions even though it was relatively unimportant in the high-calcium solutions.

Richards refers only to the different responses in succulence that occurred between the K<sub>3</sub> (medium potassium supply) and the K<sub>1</sub> (high potassium supply) treatments. Accordingly, only the results for these potassium treatments will be considered. Now, inspection of the results of Richards and Shih shows that the increase in succulence that occurred from the K<sub>3</sub> to the K<sub>1</sub> treatments was not due to sodium, calcium, or phosphorus. It must in some way have been due to the increase in potassium. Our own investigations (Phillis and Mason, 1945) have made it clear that the three cations, potassium, sodium, and calcium, all increase hydration and that they all increase it to the same extent. The sum of these three elements has consequently been plotted against hydration. The correlation diagrams for both the high (left) and the low (right) phosphorus treatments are shown in Fig. 1. The medium sodium treatment has been included and the three samples (taken after 33, 40, and 54 days) for each potassium treatment have been joined. Richard and Shih's nomenclature has been followed.

	High sodium, low calcium.	Medium sodium, medium calcium.	No sodium, high calcium.
High potassium . . .	A K <sub>1</sub>	B K <sub>1</sub>	C K <sub>1</sub>
Medium potassium . . .	A K <sub>3</sub>	B K <sub>3</sub>	C K <sub>3</sub>

The sum of the three cations will be referred to as *salt*. It must be emphasized that Richards and Shih only determined the total amounts of the three cations and not the amounts in solution in the sap (cf. Phillis and Mason, 1940). Furthermore, phosphorus is not included in this salt estimate. Some of the anomalies shown in Fig. 1 may well have been due to the inadequate way in which salt has been estimated.

Inspection of Fig. 1 will make it clear that for a given salt content, hydration was generally less in the K<sub>1</sub> treatments (big plants) than in the K<sub>3</sub> treatments (small plants). This difference is attributed to the size factor. The difference was greatest for the 'A' solutions and least for the 'C' solutions. This difference is attributed to the toxicity of the 'A' solutions. It will be seen that there was a tendency, especially in the high-phosphorus series, for hydration to diminish between the first and the third samples. This change may be largely due to salt. It will also be seen that the difference between the K<sub>1</sub> and the K<sub>3</sub> treatments tended to diminish between the first and the third samples. The diminution in hydration with age and the diminution in the size effect may be attributed to the fact that the nutrient salts were applied to the pots at the beginning of the experiment and naturally the salt content of the pots

diminished as the plants developed. The diminution in the salt content of the leaf tended to be greater at the  $K_1$  level (big plants) than at the  $K_3$  level (small plants).

To sum up, the reason for the increases in hydration that occurred as the potassium supply was increased in the high-calcium plants was due to the fact that potassium increased the salt content of the leaf and there was no important size effect. On the other hand, an increase in the salt content of

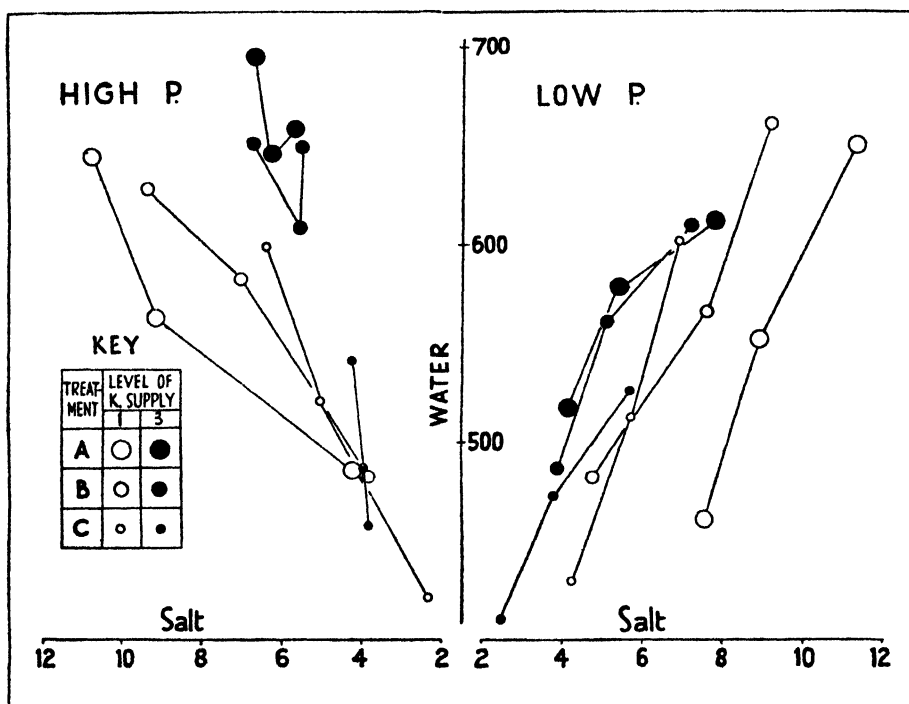


FIG. 1. The relation between salt (per 100 gm. dry weight) and foliar hydration for the  $K_1$  and  $K_3$  treatments in Richards and Shih's experiment. The three samples for each treatment are joined together, the first sample having in all cases the highest water value.

the leaf due to an increase in potassium<sup>1</sup> resulted, in the high-sodium series, in a decrease in hydration because the size factor operating in a physiologically arid solution outweighed the salt effect. That the size factor was operating, and not a difference in carbohydrate level, is apparent from the fact that there is no consistent relation between carbohydrate level and the differences between the  $K_1$  and  $K_3$  treatments (cf. Russel, 1938).

### III. THE EXPERIMENT OF PHILLIS AND MASON

Thus while we (1943) were able by increasing the supply of potassium to bring about either increases or decreases in hydration according as the physical

<sup>1</sup> Richards and Shih used sulphate of potassium, which, as James (1930) has shown, has a much smaller effect on hydration than the chloride.

environment was wet or dry, Richards and Shih succeeded in changing the direction of *hydration* by alterations in the ratio of calcium to sodium. We interpreted the results of our experiment in terms of water strain and size, while no explanation has been attempted by Richards of the results of the experiment of Richards and Shih. Richards must, however, have suspected that in our experiment potassium or some other mineral element was responsible for the increase in hydration in our wet house and that this tendency was reversed in the dry house by a high carbohydrate level, for on commenting on our work he says: 'Mason and Phillis' data are interpreted entirely along the lines of an assumed positive potassium effect combined with the size effect, and possible effects of other elements and of carbohydrate level are disregarded.'

In our (1943) experiment in which we reversed the direction of hydration according as the plants were growing under wet or dry conditions, we only published results for size (dry weight of whole plant), hydration, and electrical conductivity of sap. We also determined, however, total sugars, polysaccharides, calcium, phosphorus, protein, and of course potassium. These results (except potassium) are shown in Fig. 2, along with hydration, size and conductivity. Sodium was not determined, as cotton, unlike barley, is a low-sodium plant (cf. Collander, 1941) and only traces of this element are present (cf. Dastur and Ahad, 1941; Phillis and Mason, 1940).

Inspection of Fig. 2 makes it clear that there is no foundation for Richards's suggestion. Potassium, which is not shown on the graph, increased in both the wet and dry houses. It will be seen that the changes in labile carbohydrate and all the mineral elements determined were similar in the two houses; only hydration showed a reversal in direction. It may be added that in the bark the changes in hydration were similar to those in the leaf, while the changes in labile carbohydrate were the reverse of those in the leaf.

#### IV. THE EFFECT OF VARYING NITROGEN SUPPLY

'Finally', Richards says, 'if in pot culture a size factor is of so great importance in determining the direction of deviation in water content with potassium deficiency, why is the same factor apparently inoperative with variation in the supply of other nutrients, as nitrogen and sulphur?' The argument seems to be, if variation in the supply of elements like nitrogen which cause marked differences in size are not accompanied by hydration changes, how can the changes in hydration that accompany variation in potassium supply be due to changes in water strain? The first point to emphasize is that marked changes in the direction of hydration under varying nitrogen supply do occur.

Thus, in Fig. 3, the results for hydration, size (dry weight of whole plant) and electrical conductivity of sap are shown in an experiment in which nitrogen supply ranged from 50 to 2,500 p.p.m., and in which collections were made when the plants were 38 and 74 days old. At the time of the second collection the plants were rather heavier than in the potassium experiment described in

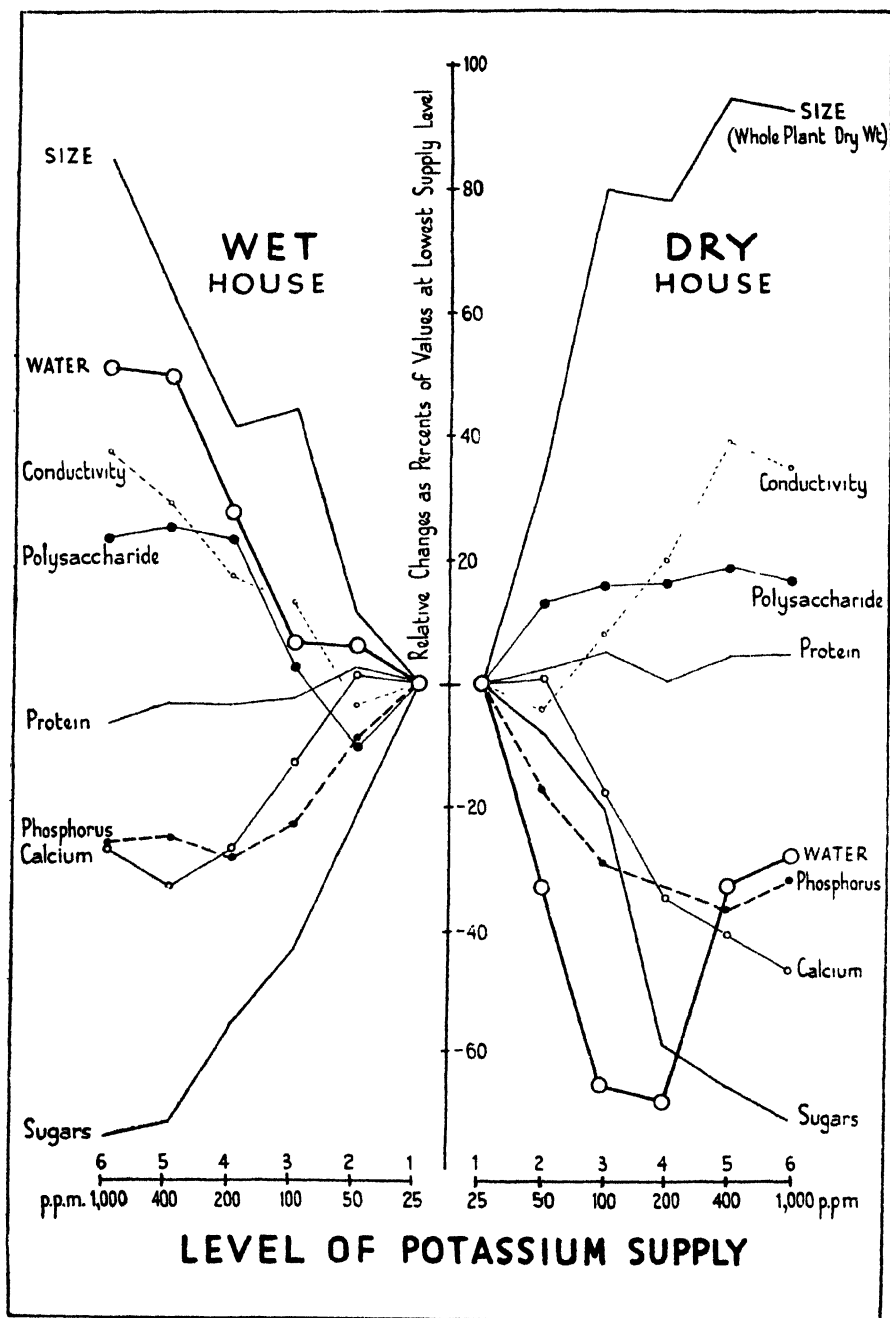


FIG. 2. Changes in size and in foliar composition with varying potassium supply under wet and dry conditions. Results are expressed as percentages of the values at the lowest level of supply. The foliar values, with the exception of conductivity, represent concentrations per 100 gm. dry matter. Conductivity values were determined on expressed sap diluted tenfold, at 0° C. The water changes are magnified tenfold.

our second paper (1943) on the size factor. The plants were grown in sand in containers similar to those used for the potassium experiment.

It will be clear from the figure that the direction of hydration was reversed between the first and second collections. It also seems clear that this change

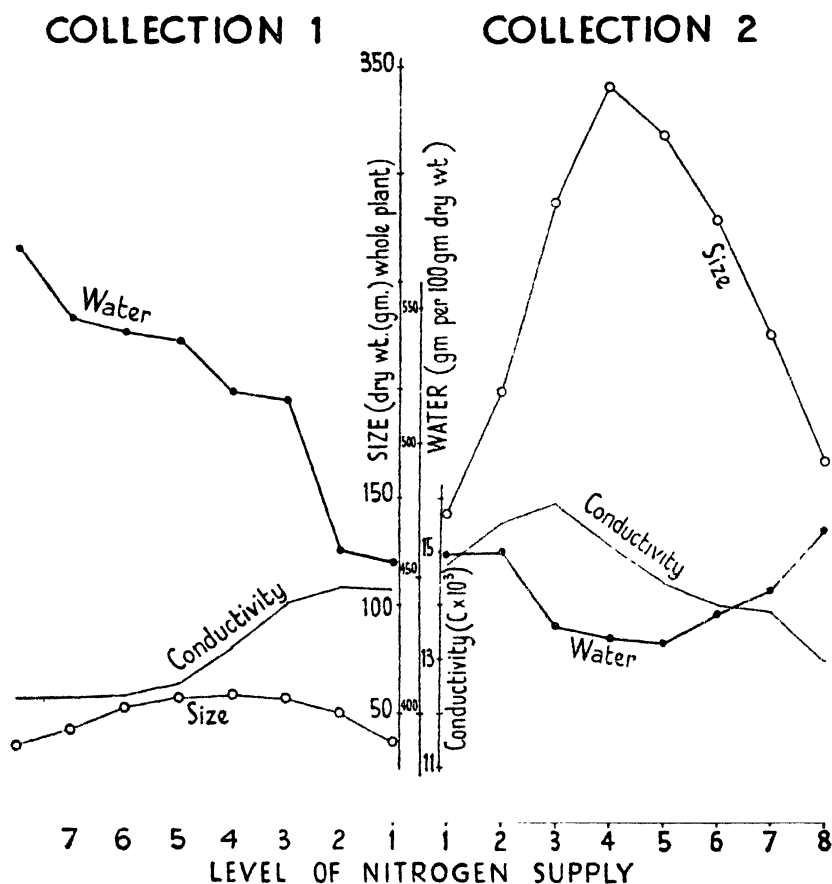


FIG. 3. The relation between foliar hydration and size at two collections (38 days and 74 days) in an experiment with varying nitrogen supply. The values for sap conductivity ( $C \times 10^3$ ) are also shown.

in hydration cannot be attributed to changes in conductivity. Moreover, it will be seen that in the second collection the pattern of hydration is the inverse of that of size. The implication is that water strain increased between the first and second collections in the bigger plants, and that this caused a reversal in the direction of hydration. It should be noted that the level of hydration in the smallest plants showed no marked change between the two collections.

The second point raised by Richards's question is that size differences need not be accompanied by hydration differences even under comparatively arid

conditions if the factor responsible for the difference in size also alters such processes as transpiring power, water-absorbing power of root, conductivity of xylem. Furthermore, even if a size difference is accompanied by a difference in water strain, there need be no appreciable change in hydration. Changes in hydration caused by changes in potassium supply under humid conditions are apparently due, at least in part, to changes in the salt content of the cell, while changes in hydration due to changes in nitrogen supply are not apparently caused by salt. To what they are due is not yet clear<sup>1</sup> (cf. Mason and Phillis, 1943; Phillis and Mason, 1945), but it is clear that the mechanism of water uptake brought about by increases in potassium and in nitrogen respectively are fundamentally different, and consequently the responses to increases in water strain may also be different.

#### V. THE AGE EFFECT

In an addendum to his paper Richards comments on our second paper on size as follows: 'The problem of the "size factor" in relation to water content and potassium supply has also been further investigated. Pronounced effects of age of plants were found on water content, even under conditions of good moisture supply, together with other effects incompatible with the "size factor" operating *via* simple water strain.' Richards is referring to the decrease in hydration that occurred between the first and second collections in the wet series. The interval between the collections was only 7 days, and as the decline in hydration was very marked in plants that showed no evidence of the size effect, I am inclined to agree with him that the change in level of hydration was not due to an increase in water strain, but it does not follow that this change is 'incompatible with the "size factor" operating *via* simple water strain'.

Richards and Shih's results show a decline in hydration (at least in the K<sub>1</sub> treatments) as the plants aged. This, as has been pointed out, can in a large measure be attributed to a decline in salt content. No such decline in salt content was exhibited by the plants of our wet treatments, so some other explanation must be sought. It will be recollected that the smallest plants of the nitrogen experiment reported in the present paper showed no sensible change in hydration between the two collections.

It is possible that the reason for the decline in the level of hydration in our wet series lies in 'flower-pruning'. The removal of flowers and bolls has, as Mason and Maskell (1930) have shown, the same effect on the export of phloem-mobile materials from the leaf as ringing the stem. Carbohydrates pile up in the leaf in abnormal amounts. The result of this would be to cause an *apparent* reduction in hydration. We use the word 'apparent' because this

<sup>1</sup> If an increase in nitrogen leads to an increase in the amount of anhydrous protoplasm and the latter to an increase in the amount of water, there will be an increase in the amount of water but no increase in the hydration of protoplasm. But if the increase in dry weight is less than that of anhydrous protoplasm, there will be an increase in hydration in terms of dry weight. Addition of nitrogen may also of course improve in some way the water economy of the plant and so increase protoplasmic hydration.



need not be accompanied by any real change in protoplasmic hydration (cf. Mason and Phillis, 1943).

This raises an important issue in experiments in relation to hydration carried out on the intact plant. We (1939) have shown that an increase in the supply of potassium causes not only an increase in the dry weight of the whole plant, but that the increase in the leaf is less than that in the stem, roots, &c. From this we inferred that potassium promotes export from the leaf. Such a change in *distribution* might therefore lead to an apparent increase in hydration. Moreover, the reduction in the proportion of leaf that occurs when potassium is increased might lead to a reduction in water strain and this might cause an increase in hydration. These changes in distribution therefore provide *one* reason why experiments made by means of our leaf-disc technique, in which water can be expressed on the sample basis and in which the size factor is relatively unimportant, are to be preferred to those carried out on leaves attached to the plant. The disc technique can, unfortunately, only be used with fairly mature leaves, so that where investigations relate to the effect of mineral supply on embryonic tissues, recourse must still be had to the intact plant.

## VI. CELL SIZE

Richards has stressed the possibility 'that carbohydrate status, with effects on wall extensibility, as envisaged by Richards and Shih may well be a dominant constituent of the size effect'. Richards and Shih attributed the absence of a potassium effect on hydration as probably due to the correlation existing between potassium and carbohydrate level. Carbohydrate by restricting extensibility should tend to restrict cell size. Richards and Shih did not themselves determine carbohydrate, but utilized the results of an earlier investigation by Russel (1938). Examination of Russel's carbohydrate data and Richards and Shih's potassium data does not, however, reveal any significant correlation between the two. Under the circumstances, the carbohydrate hypothesis does not rest on any adequate foundation.

The situation is probably correctly summarized by Yapp (Maximov, 1929), who says: 'the water factor affects mature structure to a great extent mechanically, by influencing the degree of expansion of the individual cells and therefore indirectly the thickness of their walls. Hence the smaller size of the cells under dry conditions.' The size factor by increasing water strain will thus restrict the size of the cell, diminish the amount of water in it, and reduce protoplasmic hydration. It also, as we have shown, leads to a reduction in hydration, when hydration is expressed either on the dry weight or on the protein basis.

Warne (1936) found that an increase in potassium supply resulted in increased growth, which was reflected in an increased number of leaves on the plant and in the production of larger leaves. The increase in leaf size he attributes to an increase in the size of the individual cells. Thus, an increase in the supply of potash in his experiments resulted in larger cells, more water

per cell, and usually to an increase in hydration when hydration was expressed on the dry-weight basis. Richards and Shih, on the other hand, who found no consistent effect of potash on hydration, state that in the type of plant investigated by them 'cell size is almost certainly not increased by addition of potassium and may be decreased'. In their experiment it would seem (at least in the high-sodium treatment) that the increase in salt due to potassium tended to increase hydration, but that the size effect was so emphatic that it neutralized and reversed this tendency.

## VII. SUMMARY

1. Richards's criticisms of the size hypothesis are examined.
2. It is pointed out that some of the nutrient solutions employed by Richards and Shih were probably toxic and consequently physiologically arid.
3. It is shown that there are strong indications that the size factor was operating in the plants grown in these solutions.
4. Data are presented which prove that the reversal in hydration found by Phillis and Mason cannot have been caused by differences in the mineral or carbohydrate levels of the leaves.
5. It is also demonstrated that the size factor may operate when plants are grown under varying nitrogen supply.

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# Studies in the Vegetative Growth and Anatomy of the Tea Plant (*Camellia thea* Link.)<sup>1</sup>

## III. Apical Activity and the Flush Cycle in relation to Manuring

BY

T. E. T. BOND

(*Tea Research Institute of Ceylon, St. Coombs, Talawakelle, Ceylon*)

### INTRODUCTION

AN opportunity of investigating in tea the effects of manuring on apical activity and the flush cycle (Bond, 1945) was afforded by the pruning in April 1943 of one of the Tea Research Institute's long-standing manurial experiments. The experiment (Eden, 1935, 1944) is a factorial one involving duplicates of all possible combinations of three levels each of nitrogen, phosphate, and potash; the levels are as follows: N<sub>40</sub>, N<sub>60</sub>, N<sub>80</sub>; P<sub>0</sub>, P<sub>30</sub>, P<sub>60</sub>; K<sub>0</sub>, K<sub>20</sub>, K<sub>40</sub> in lb. per acre. Thus, there are 54 plots, and the comparison between individual manurial levels is based on an eighteen-fold replication. The observations here described were made on 54 bushes, one from each plot, which were chosen at 'tipping' time (Eden, 1931) in October 1943 and left untipped and out of plucking until they were again pruned down in November 1944.

The bushes were not chosen at random; they were as far as possible of uniform type, representing the majority of the tea in the experimental area, and similar in appearance to those previously studied (Bond, 1942). Only bushes towards the centre of the plots and adjacent on all sides to other mature bushes were included.

On each bush 10 shoots were labelled for the detailed study of apical activity. They were marked as they became available during a period of two months from the middle of December 1943, choosing only lateral shoots which had completed at least a single flush cycle and in which the *banji* bud was newly exposed. The subsequent development of these buds was recorded at fortnightly intervals over a total period of 21 weeks for each shoot, marking according to the stages in the flush cycle as previously recognized (Bond 1945), i.e. beginning with stage *A*. The appendage values (Bond, 1945) for the several stages were determined from averages of 50 shoots or buds collected from among the labelled bushes at the successive records, checked, for stage *A* (4.3 appendages), by a further 25 buds of the second cycle. A high degree of consistency was obtained, the difference between stage values averaging 0.75 appendages, as compared with an average standard error for the means

<sup>1</sup> Owing to the widening scope of the investigations, the words 'with Special Reference to the Phloem' are now omitted from the title of this series.

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for stages of 0.095. From the fortnightly determinations of the average appendage values the regression coefficients of appendages on time were calculated as an index of apical activity. As an alternative, the total number of appendages at the end of 21 weeks was obtained by direct observation and dissection of the apical buds.

At various times small numbers of shoots were removed from the labelled bushes for analysis of the flush cycle and for comparison with other shoot samples taken from the plots as a whole. The data were completed by recording the total weight of prunings, and the weight of the shoots removed at an early 'tipping' in February 1945.

## RESULTS

(1) *Apical activity.* The regressions for appendages on time again emphasized the continuity of apical activity (Bond, 1945); giving always a very close fit to a linear relationship. The average plastochron for the treatment means of 18 bushes varied from 3.3 to 4.0 weeks, with a general mean of  $3.63 \pm .17$  weeks. Similar values as determined from the observed totals of appendages produced at the end of the 21 weeks varied from 3.0 to 3.6 weeks, with a general mean of  $3.31 \pm .11$  weeks, not significantly different from the preceding. A summary of these data is given in Table I (random selections of 7 shoots only were available on account of incidental loss and injury).

TABLE I  
*Total Appendages produced in 21 Weeks*  
(Treatment means of 7 shoots from each of 18 bushes)

Manurial levels								
N <sub>40</sub>	N <sub>60</sub>	N <sub>80</sub>	P <sub>0</sub>	P <sub>30</sub>	P <sub>60</sub>	K <sub>0</sub>	K <sub>20</sub>	K <sub>40</sub>
6.23	6.87	5.96	6.74	6.31	6.01	6.93	5.90	6.22
General mean: 6.35 (plastochron of $3.31 \pm .11$ weeks)								
S.E. of treatment means: 0.38 (6.0%)								

The results show no significant difference as between the different manurial levels, but the general tendency to a negative effect, especially for phosphate, should be noted. The apparent inverse relationship between the effects of nitrogen and of potash is of interest in view of the fact that a significant NK interaction was obtained at  $P < 0.05$ .

The mean value of 6.35 appendages represents rather more than one complete flush cycle on the average. However, there was considerable variation in this respect, some buds even remaining unexpanded at stage *D*, others reaching stage *E*, or the expansion of the first scale leaf of their third cycle (l.c.). No significance was obtained whether the data were considered in terms of appendages *expanded* (as distinct from *total* appendages as in Table I) or of flush cycles completed or in progress, although they were quite consistent in showing a maximum at the N<sub>60</sub> level and a marked negative tendency for both the P- and K-effects.

(2) *Composition and dimensions of the flush cycle.* The first flush cycle produced by the unfolding of the newly exposed terminal buds on the labelled shoots had an average length of 6.7 cm. and comprised 5.3 expanded appendages, of which 2.2 were the basal scale leaves. The shoots which had developed at least as far as this stage being unevenly distributed, a separate sample of 20 lateral shoots in their second cycle was taken from each bush for an analysis of the manurial effects as shown in Table II.

TABLE II  
*Composition of the Flush Cycle*  
(Treatment means of 20 shoots from each of 18 bushes)

Type of appendage.	Manurial levels.									
	N <sub>40</sub>	N <sub>60</sub>	N <sub>80</sub>	P <sub>0</sub>	P <sub>30</sub>	P <sub>60</sub>	K <sub>0</sub>	K <sub>20</sub>	K <sub>40</sub>	
Scale leaves . . . . .	2.10	1.90	2.00	2.06	2.09	1.86	2.21	1.88	1.92	
Fish and flush leaves	3.41	3.13	3.38	3.31	3.39	3.21	3.31	3.21	3.39	
Total. . . . .	5.51	5.03	5.38	5.37	5.48	5.07	5.52	5.09	5.31	

General mean (total appendages): 5.30 (= 2.00 + 3.30)  
S.E. of treatment means: 0.12 (2.3%)

As far as the total content of appendages is concerned, the minimum value for the N<sub>60</sub> treatment is significantly different from the mean of the N<sub>40</sub> and N<sub>80</sub> values at  $P < 0.01$ , while significance at  $P < 0.05$  is similarly attained by the minimum and maximum values respectively for P<sub>60</sub> and for K<sub>0</sub>. There is again evidence here of a negative effect for phosphatic and potash manuring. However, from mixed, 20-shoot samples taken at random from the plots as a whole (comprising on the average 5.42 appendages including 1.91 scale leaves) there were no significant differences at all between manurial treatments, despite the fact that the standard error was here reduced to 1.1 per cent.

The proportion of scale leaves and foliage leaves within the cycle is of interest in view of the possibility of nutritional control envisaged in the previous paper. However, examination of all the data available, from 400 shoots, gave no significant correlation between them ( $r = +0.0808$ ).

The dimensions of the flush cycle were studied from measurements of the cycle which had just completed its development at the beginning of these observations, when the labelled shoots were first selected. Perhaps because of the fact that it had developed early in the period of recovery from pruning, when growth is known to be vigorous, the mean length was relatively high, i.e. 9.8 cm. With a standard error for treatment means of 6.3 per cent. only the nitrogen effect was significant, with a positive (and almost linear) regression from 8.8 cm. at N<sub>40</sub> to 11.0 cm. at N<sub>80</sub>, with  $P < 0.02$ . It should be noted that these dimensions include a varying number of flush, fish, and scale internodes, for the separate treatment of which much further data would be necessary.

(3) *Growth weight.* The relative growth weights under the different treatments as given by the total weight of prunings after 19 months, are shown in

Table III. With the relatively high standard error of 8.05 per cent. of the treatment means, only the nitrogen effect reaches significance, at  $P < 0.05$ , although the minimum value at the  $N_{40}$  level differs from the mean of the

TABLE III  
*Growth Weights in 19 Months (as percentages)*  
(100% = 7.17 lb. dry wt. prunings, per bush)

Manurial levels								
$N_{40}$	$N_{60}$	$N_{80}$	$P_0$	$P_{30}$	$P_{60}$	$K_0$	$K_{20}$	$K_{40}$
79.18	111.71	109.12	94.90	110.16	94.94	91.03	102.46	106.52
S.E. of treatment means (of 18 bushes): 8.05%								

other two at  $P < 0.01$ . The absence of a significant response to phosphate and potash differences is not surprising in view of Eden's (1935, 1944) results, but the 'curvature' in the response to nitrogen is in marked contrast to the uniformly linear, proportional responses to this element obtained from this same experiment and others by Eden, and demonstrated also from North India by Cooper (1939).

(4) *Correlation between growth weight and apical activity.* No correlation was found between total growth as measured by the weight of prunings and the rate of apical activity. The values of  $r$  obtained, namely  $-0.0900$  with the total appendages in 21 weeks and  $+0.0079$  with the regression data (in both,  $n = 52$ ), were negligible. A reason for this lack of correlation is indicated by the analysis of covariance as in Table IV, whence it appears that there is a differential effect as between nitrogen levels on the one hand, for which the correlation is positive, and levels of phosphate and potash on the other hand, for which it is negative. Being based on a single degree of freedom only, the value of  $r = -0.9974$  for the potash levels alone reaches significance at  $P < 0.05$ . The relatively high value of  $r = -0.5321$  for the NK interaction is suggestive in view of the significance attained by this component in the analysis of the data for total appendages, noted above.

TABLE IV

*Correlations between Total Yield (lb. of dry wt. prunings) and Rate of Apical Activity (regression coefficient,  $\times 100$ , for appendages on weeks)*

	Degrees freedom.	Sums of squares.		Sums of products.	Value of $r$ .
		Yield.	Apical activity.		
Blocks . . .	5	24.876	206.3	-23.307	-0.3253
Nitrogen levels	2	60.538	221.2	100.238	+0.8662
Phosphate „	2	14.337	50.7	-17.171	-0.6369
Potash „	2	11.947	236.3	-52.996	-0.9974 ( $P < 0.05$ )
$N \times P$ . . .	4	17.421	278.7	3.531	+0.0507
$N \times K$ . . .	4	43.602	455.2	-74.961	-0.5321
$P \times K$ . . .	2*	19.509	33.9	1.662	+0.0646
Error . . .	32	192.148	2861.1	73.186	+0.0987
Total . . .	53	384.378	4343.4	10.182	0.0079

\* Two degrees of freedom confounded.

No significant correlations were demonstrated between growth weight and the composition and dimensions of the flush cycle.

#### DISCUSSION

The suggestion was previously made by the writer (1945) that the flush cycle in tea might depend on the maintenance of a level of nutrition insufficient for continuous flushing but in excess of that required merely for the continuous production of scale leaves. On that view, it is to be regarded as the result of a fluctuation in the rate of apical activity about a position of unstable equilibrium represented by the average value of the plastochron for the cycle as a whole. The speed of fluctuation, for a given average plastochron, will control the number of appendages in the cycle, but no correlation was found between this and the rate of apical activity ( $r = +0.0656$ ).

The differences in manurial level and soil fertility occurring in the present investigations have in no case been sufficient to cause any departure from the normal flushing behaviour. The smallness of the observed effects possibly indicates that the fluctuation in apical activity, once established, is not easily modified until the conditions are such that a permanent shift is produced, in one direction or the other.

On the whole, phosphate and potash have had a negative effect both on apical activity and on the number of appendages in the cycle. In other words, they have tended to cause a lengthening of the plastochron and, relatively, a shortening of the cycle. This would appear, on the hypothesis previously put forward, to be associated with a more rapid expansion and earlier lignification of the parts laid down which, in turn, would contribute to the negative correlations observed between growth rate and apical activity.

The effect of nitrogen has been rather more pronounced, although even here the degree of significance attained has not been entirely satisfactory. Prominent in all the responses, except for the linear effect on the dimensions of the flush cycle, has been the curvature at the  $N_{60}$  level. With this amount of nitrogen, the shortest average plastochron has coincided with the shortest period of fluctuation in apical activity to produce a flush cycle with fewest leaves and relatively long internodes. Total growth weight here appears to be a function of both the apical activity rate and the rate of subsequent expansion and the importance of nitrogen as affecting meristematic activity at the apex previously foreshadowed, is confirmed.

The departure from linearity in the nitrogen response, which is so unusual in tea, may be due to the fact that the bushes were not in plucking, but against this must be noted the fact that an even more pronounced curvature in the response (although not significant) was obtained from the early 'tipping' yields, subsequent to pruning. A more probable explanation is simply that the bushes were not as fully representative of the tea as a whole as they were intended to be: the variability of the crop being as great as it is the only justification for working with single bushes was the high degree of replication for manurial levels allowed by the design of the experiment. Thus, both the

abnormal response to nitrogen and the, in general, low degree of precision obtained may be due to this same cause. Future work with uniform, clonal material will be needed for any really adequate confirmation of the nutritional effects here tentatively put forward.

# SUMMARY

Apical activity and the composition and dimensions of the flush cycle as affected by manuring were studied on 54 unplucked tea bushes from a factorial NPK experiment comprising duplicate treatments of all combinations of the three manures at three different rates of application. The total growth of the bushes during a period of 19 months from pruning was also measured.

The effects observed were relatively slight. Both phosphate and potash tended to reduce the rate of apical activity and to cause a decrease in the number of appendages per cycle. The effects of nitrogen were seen chiefly at the middle level of application (at 60 lb. N per acre) which gave the highest growth-weight, the highest rate of apical activity, and the greatest number of leaves in the cycle. Thus, yield and apical activity appear to be positively correlated for differences in the level of nitrogen, negatively correlated for differences in phosphate and potash.

These observations were only made possible through the kindness of Dr. T. Eden in giving me access to his manurial experiment and in placing the resources of his Department at my disposal.

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# Secondary Association and Heterochromatic Attraction

## I. *Cicer arietinum*

BY

P. T. THOMAS

AND

S. H. REVELL

(*John Innes Horticultural Institution*)

With Plate V and one Figure in the Text

### INTRODUCTION

THE mechanism of the attraction between secondarily paired bivalents at metaphase of meiosis has never been satisfactorily explained. This is probably because this type of association has only been observed where the chromosomes are small and the material consequently unsuitable for critical study. Darlington and Moffet (1930), who first used the term 'secondary association', considered it to be due to a residual attraction between more distantly related chromosomes. Heilborn (1936), on the other hand, considered that this uneven distribution of bivalents was merely a pattern resulting from the operation of unequal forces of repulsion.

Recent observations on a number of plants with small chromosomes have suggested that the formation of chromocentres by the fusion of heterochromatin during the prophase of meiosis is related to the association of bivalents at later stages. Thus chromocentres have been observed in all plants which exhibit secondary association, but not in those like *Morus nigra* ( $2n=308$ ) which show little or no secondary association (Thomas, unpub.). In addition, the observations of Bolle and Straube (1941) that heterochromatic regions in *Impatiens* have an even stronger pairing affinity than homologous euchromatic regions seemed to support this hypothesis.

The present paper describes our observations on a diploid and a colchicine-induced autotetraploid of *Cicer arietinum*.

### MATERIALS AND METHODS

The original samples of *Cicer arietinum* were obtained by Dr. C. D. Darlington in order to corroborate earlier reports by Dixit (1932) and others of the existence of a *gigas* form with 16 chromosomes as a mutant of the normal 14-chromosome type. We are indebted to Mr. L. G. Wigan for seeds of the two supposed types as well as seeds from hybrids and selfed plants. The two types undoubtedly differed genetically, but only chromosome numbers of 16 were recorded, although an extra fragment was sometimes seen.

Meiosis was studied after fixation in 3:1 alcohol/carmine-iron-acetic acid, and stained in half-strength aceto-carmine (Thomas, 1940). Some material was also stained in La Cour's aceto-lacmoid with good results (Darlington and La Cour, 1942).

Iyengar (1939) has described the cytology of *Cicer arietinum*, using sectioned material stained in crystal violet. In our opinion this technique is quite unsuitable for the material because, as his photographs show, the nuclei become so contracted that observation at any stage is difficult, and attempts at analysis of the secondary association of bivalents at metaphase can be of little value. We have found the method described to be much more favourable for all stages.

#### THE CHROMOSOMES

Aceto-carmine squashes of root-tips show that the nucleolus is often persistent during mitosis: it fails to dissolve completely and is carried on to the metaphase plate. In many divisions also, regions of the chromosomes are starved of nucleic acid—a natural demonstration of heterochromatin. For these reasons second anaphase of meiosis was found to be the best stage for determining chromosome morphology (see Text-fig.). It will be seen that although all the centromeres are median or submedian, the size range makes it possible to recognize three of the eight chromosomes, the two longest and the shortest ones, individually. The remaining five are of intermediate size and cannot be distinguished from one another.

#### *Meiosis in the diploid*

Bolle and Straube (1941), in their account of meiotic prophase in *Impatiens*, describe a marked precocity of primary pairing in heterochromatic regions. This is followed at pachytene by another, secondary, fusion of heterochromatin. These stages can be observed under very favourable conditions in *Cicer*. Primary pairing at zygotene is intermittent, but although heterochromatin is clearly visible it does not always pair before the euchromatin (Pl. V, Fig. 1). The secondary fusion of heterochromatin is, however, very evident (Pl. V, Fig. 2), and by late pachytene it is shown in varying degrees by all nuclei. That it is a genuine secondary fusion is certain, because it clearly occurs between chromosomes that are already homologously paired.

Diplotene in *Cicer* is of the diffuse type and closely resembles the condition described in detail by Matsuura (1935) in *Mitrastemon*. Except for small localized regions which remain condensed, the bivalents lose their nucleic acid charge, and the protein framework is diffuse. Our attempts to demonstrate the continuity of the pachytene fusions through this stage were necessarily confined to those cells which showed only moderate diffuseness. In such cells interbivalent connexions can be clearly seen. These connexions are often as definite as the connexions seen at a terminal chiasma. For example in Pl. V, Fig. 4, the connexion between the two bivalents at 7 o'clock may be compared with the simple large bivalent at 12 o'clock. Occasionally the bivalents are held closely together, but usually the surface repulsion is sufficient to force

them apart: the connexion is evidently capable of stretching. In some early diplotene nuclei numbers of small nucleoli were seen in association with each of the bivalents (Pl. V, Fig. 3).

Diakinesis is short. The bivalents become very condensed, and in nearly every nucleus interbivalent connexions, often greatly attenuated, are plainly visible. A marked prometaphase occurs during spindle formation.

At metaphase secondary association is pronounced. In side views the three pairs of chromosomes that are identifiable at second anaphase can now be recognized as three bivalents. We have called the two large bivalents  $L_1$  and  $L_2$ , and the small one  $S$  (see Text-fig.);  $S$  is easily recognized by its small size.  $L_1$  and  $L_2$  can be distinguished by the length of the arm in which chiasma formation occurs: this is longer in  $L_1$ . The co-orientation position of the centromeres is therefore different in the two cases, and the degree of terminalization of the respective chiasmata is also affected. Thus in Pl. V, Fig. 6,  $L_1$  is on the left and  $L_2$  on the right; in Pl. V, Fig. 7,  $L_1$  is third from the left and  $L_2$  on the right.



Second anaphase of meiosis showing the three identifiable chromosomes,  $L_1$ ,  $L_2$ , and  $S$ . ( $\times 1,000$ .)

It was therefore decided to attempt an analysis of secondary association from side views instead of from polar views, with the object of estimating the frequency of association of these three bivalents with one another and with the five others. Tests were made to observe the effect of squashing on bivalent grouping: examination of many individual cells before and after pressure of the cover-slip showed that the association in fixed unsquashed material was not disturbed by squashing. The technique was therefore considered to be reliable, and the result of an analysis of 50 cells is given in the table. Many metaphases showed such a high degree of association that they could not be interpreted: it should therefore be emphasized that these cells are selected for low grouping. A clear qualitative demonstration is given, however, that pairing is possible between the three identifiable bivalents  $L_1$ ,  $L_2$ , and  $S$ , and between them and the five medium bivalents, in the combinations shown. The percentages in the table give the frequencies of different associations, and refer to the bivalents on the left. Thus 32 per cent. of the  $L_1$  bivalents are associated with  $M$  bivalents, and 56.5 per cent. of  $M$  bivalents are associated with one another.

### *Meiosis in the autotetraploid*

In the autotetraploids that were obtained by the colchicine technique it was immediately evident that, apart from the occurrence of multivalents (1-3 quadrivalents) resulting from primary pairing, the character of the secondary association was also different from that shown by the diploid. An analysis of grouping was not made, but it was estimated that in 75 per cent. of cases the recognizable bivalents were associated with their respective homologues. One

division is shown in Pl. V, Fig. 10. In this case all the six bivalents are grouped in pairs, the  $L_1$  and  $S$  pairs on the left and the  $L_2$  pair in the middle.

Table showing the Character and Frequency of Secondary Association at Metaphase in *Cicer arietinum*. (The percentages refer to the bivalents on the left of the table)

	$L_2$	$S$	$M_{1-5}$
$L_1$	1 $L_1L_2$ 2%	1 $L_1S$ 2%	14 $L_1M$ 2 $L_1MM$ 32%
	$L_2$	3 $L_2S$ 6%	15 $L_2M$ 3 $L_2MM$ 1 $L_2MS$ 38%
		$M_{1-5}$	35 $MM$ 7 $MMM$ 1 $MMMM$ 2 $L_1MM$ 3 $L_2MM$ 4 $SMM$ } A total of 113 $M$ associations 56.5%
	$S$		16 $SM$ 4 $SMM$ 1 $SML_2$ 42%

### CONCLUSIONS

We conclude from our observations that the fusion between heterochromatic regions at pachytene is directly responsible for the characteristic secondary association at metaphase. Admittedly, the evidence for a persistent connexion between the affected bivalents throughout meiosis is not entirely satisfactory in *Cicer arietinum*. Owing to the extreme detachment of nucleic acid at late prophase the interbivalent connexions could only be observed in nuclei which were less diffuse than usual. Nevertheless, confirmatory evidence from work on other plants (for example on the turnip), which will be published later, leave us in no doubt that the connexion is a real one.

The three recognizable bivalents can associate in all combinations, and also each of them can pair with the remaining bivalents. Thus we see that the fusion of heterochromatic regions is not specific. That the fusion is not entirely at random, however, is shown by the high degree of association which we observe among the morphologically similar bivalents. There is thus some preferential secondary pairing among like bivalents in the diploid, but it is not sufficient to evaluate the degree of relationship between them.

In the tetraploid, on the other hand, the situation is quite different. There is a marked preference for secondary association between homologous bivalents. It is estimated that secondary pairing between like bivalents occurs in 75 per cent. of the cases.

What, then, are the factors which make the diploid and tetraploid behave so differently? Clearly the main factor is that which determines the relative positions of the chromosome threads at early prophase of meiosis. In the diploid the heterochromatic fusions at pachytene are indiscriminate. In the tetraploid we have the regular orientation of the chromosome strands in fours to form eight potential quadrivalents. This positioning of four homologous strands at zygotene therefore affords opportunity for 'specific' fusion of heterochromatic regions at pachytene. Moreover, this preferential pairing in the tetraploid greatly reduces the frequency of association between dissimilar bivalents.

Thus in this particular tetraploid we can consider that secondary association is almost exclusively between homologous chromosomes. But in the diploid the heterochromatic fusion is largely indiscriminate, and therefore the association at metaphase can give little indication of homology. Heterochromatic fusion is non-specific in both cases, but in the tetraploid the specificity of primary pairing does not allow the indiscriminate fusion which is possible in the diploid.

#### SUMMARY

No difference in the chromosome number of a normal and a *gigas* type of *Cicer arietinum*, reputed to have 14 and 16 chromosomes respectively, could be observed. Both types, although genetically distinct, had 16 chromosomes.

The chromosome complement of both types were morphologically similar, but an extra fragment was seen at meiosis in some plants of the so-called normal type. The haploid chromosome complement in *Cicer arietinum* consists of two large chromosomes which can be distinguished from one another, five medium and one small chromosome.

At meiosis eight bivalents are formed, of which the two large and the small bivalent can be recognized individually.

Primary chromosome pairing at zygotene is intermittent at first, but the first points of contact are not always the heterochromatic regions, as Bolle and Straube discovered in *Impatiens*.

At pachytene fusions between highly charged heterochromatic regions from different bivalents is observed, and it is considered that this pairing is the direct cause of the characteristic interbivalent or secondary association at metaphase.

The intermediate stages in *Cicer* are somewhat obscured by the extreme detachment of nucleic acid from parts of the chromosomes during early diakinesis—the diffuse stage.

The association of bivalents can still be observed at this stage, however. Thus the connexions which arise by fusion at pachytene are not severed by the detachment of nucleic acid or by the interbivalent repulsion.

At prometaphase the decrease in interbivalent repulsion and reattachment of nucleic acid allows the close association of the bivalents to become re-established.

The association at metaphase is so close that the bivalent groups often resemble primarily paired multivalents.

Secondary association shows no specificity. The three recognizable bivalents can associate with one another and with the five remaining bivalents. On the other hand, a quantitative analysis shows some preferential pairing—there is a higher degree of association between the morphologically similar bivalents.

In an artificially induced tetraploid there are one to three primarily paired multivalents in addition to the bivalents secondarily paired. The tetraploid shows about 75 per cent. of homologous secondary association among the recognizable bivalents. This high specificity in the tetraploid must be due to the opportunity for fusion afforded by the orientation of homologous chromosomes in fours, at zygotene or earlier, to form potential quadrivalents.

Thus in the diploid, analysis of secondary association need not indicate chromosome relationship, whereas in the tetraploid it may do so, depending on (a) the degree of prezygotene orientation, and (b) the amount and distribution of heterochromatin.

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#### DESCRIPTION OF PLATE V

Illustrating Dr. P. T. Thomas and Mr. S. H. Revell's paper on Secondary Association and Heterochromatic Attraction I. *Cicer arietinum*.

Fig. 1. Primary pairing at zygotene.

Fig. 2. Pachytene, showing fusion between heterochromatic regions.

Fig. 3. Early diplotene, showing diffuse condition of the bivalents and the formation of accessory nucleoli.

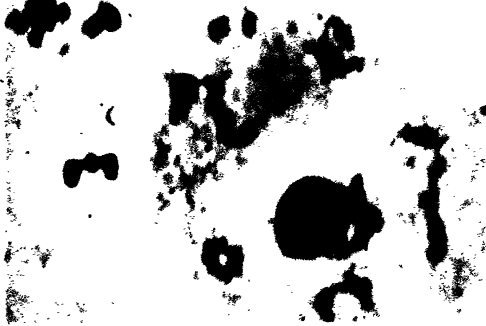
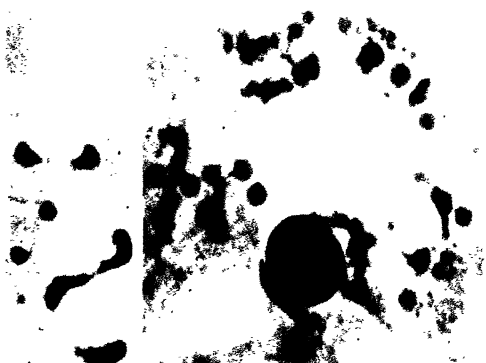
Fig. 4. Early diplotene, showing secondary association of bivalents at 3 o'clock and 7 o'clock.

Fig. 5. Metaphase, showing connexions between associated bivalents.

Figs. 6-9. Examples of secondary association at metaphase in the diploid.

Fig. 10. Metaphase in the autotetraploid, showing secondary association between homologous bivalents.









# Endosperm in *Hypericum mysorens* Heyne

BY

B. G. L. SWAMY

(Bangalore, S. India)

With fourteen Figures in the Text

THE exact mode of development of the endosperm in *Hypericum* has been a subject of some controversy. Schnarf (1914), who investigated *H. perforatum*, *H. maculatum*, and *H. calycinum*, concluded it to be of the nuclear type, but with a multinucleate 'Basalapparat' differentiated at the chalazal end of the embryo sac so as to give it a distinctive appearance. Palm (1922), on the other hand, working with *H. japonicum*, stated that after the first division of the primary endosperm nucleus one of the daughter nuclei passes down to the lower end of the sac while the other continues to divide and give rise to a large number of nuclei. The chalazal nucleus becomes invested with dense cytoplasm and subsequently gives rise to a multinucleate structure similar to that noted by Schnarf.

Dahlgren (1923) refers to this 'specially differentiated endosperm area' observed by Schnarf and by Palm, and by himself in another species, *H. kalmianum*, and considered Palm's explanation to be the correct one. He adds, however: 'It does not appear from Palm's description whether the antipodal endosperm nucleus lies from the outset in a separate cell, or whether it is only later that such a basal, though naked, cell develops. . . . Should we here have a case of the basal—probably naked—endosperm cell not developing immediately after the first division of the secondary embryo sac nucleus, we could hardly consider it a Helobiae-endosperm in the strictest sense.'

The most important paper on *Hypericum* is by Stenar (1938), who gave a detailed account of the endosperm development in *H. acutum* and rejected Palm's view that the endosperm is of the Helobiales type.

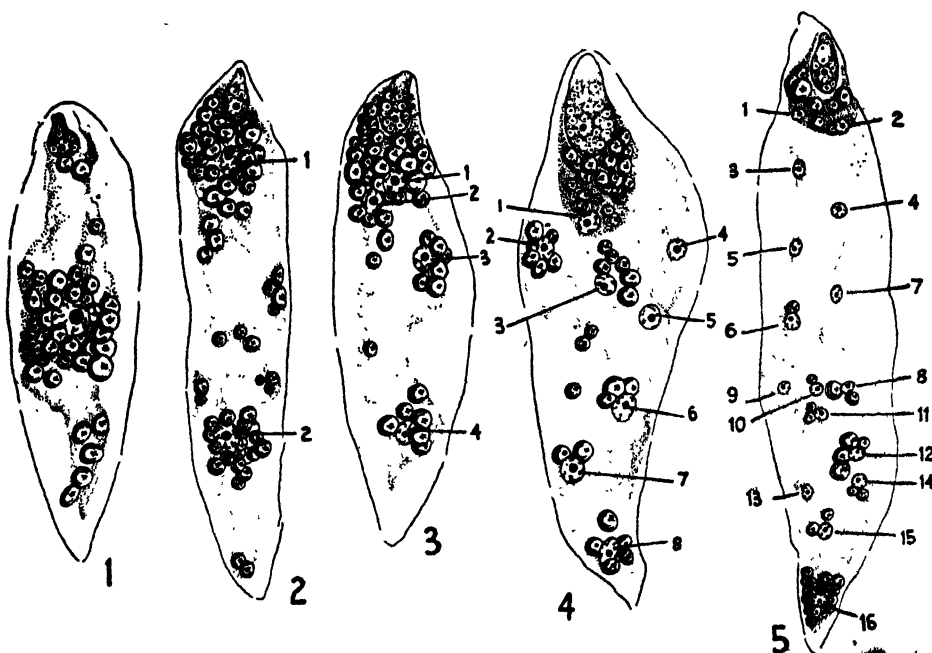
The material for the present study was collected from Nandi Hills (Mysore State) and prepared according to customary methods.

## OBSERVATIONS

The primary endosperm nucleus, prior to its first division, is seen in the central region of the fertilized embryo sac amid a mass of starch grains, the zygote lying in a relatively clear area at the micropylar end (Fig. 1). After the first division of the primary endosperm nucleus the two daughter nuclei move apart to opposite poles, carrying with them considerable quantities of

starch (Fig. 2). It is to be noted that no wall, not even a plasma membrane, accompanies this division.

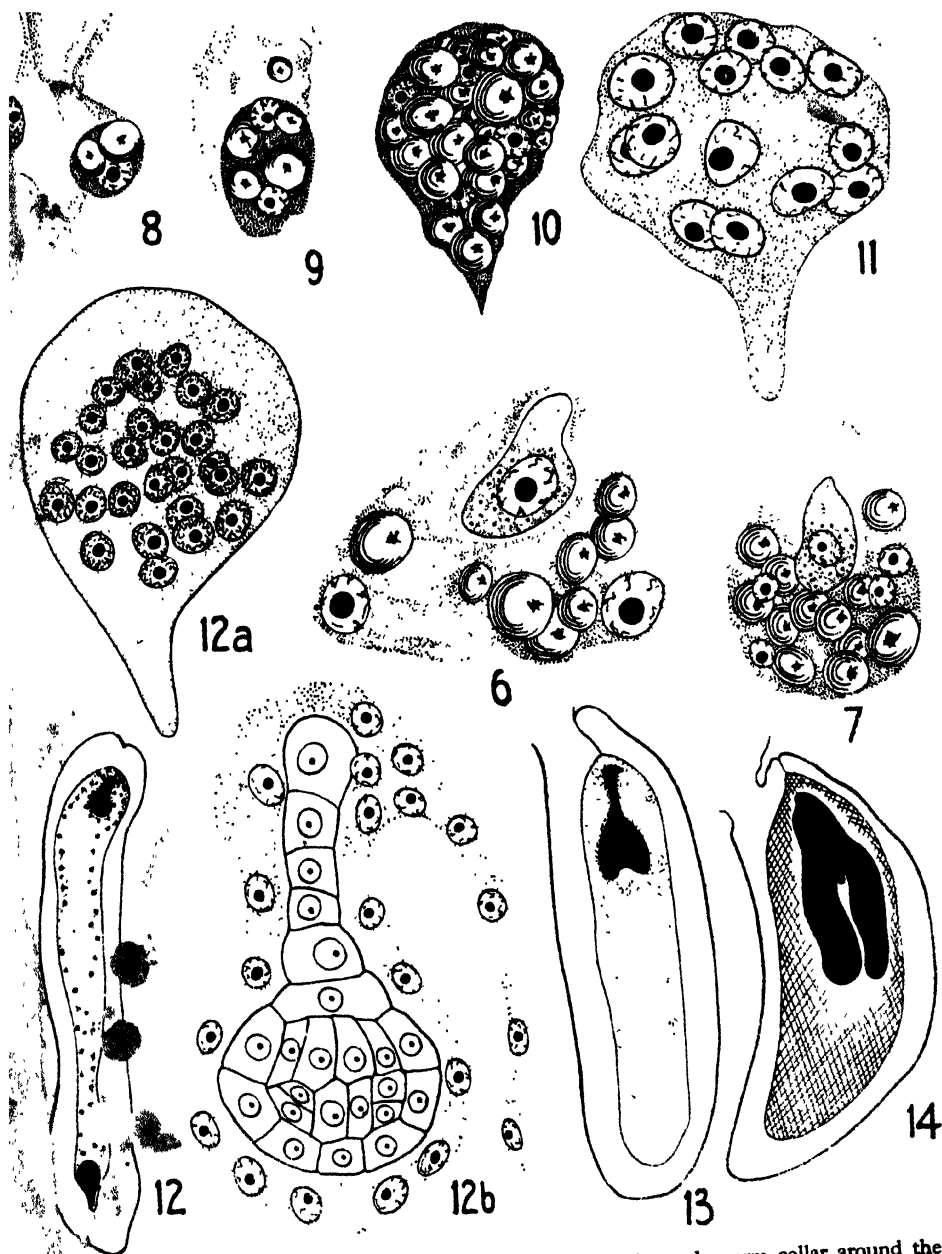
The two nuclei next divide to form four (Fig. 3) and then eight (Fig. 4). At this stage a nucleus situated near the micropylar end moves up towards the zygote with a considerable accumulation of starch grains around it, and the cytoplasm in this region becomes denser and forms a conspicuous sheath enclosing the nucleus and the starch grains (Fig. 4). The daughter nuclei



FIGS. 1-5 ( $\times 110$ ). Fig. 1. Embryo sac showing primary endosperm nucleus and zygote. Note the starch grains around the former. Fig. 2. Two-nucleate endosperm. Fig. 3. Four-nucleate stage. Fig. 4. Eight-nucleate stage; note that one nucleus (numbered 1) together with several starch grains becomes embedded in the dense cytoplasm adjacent to the zygote. Fig. 5. Sixteen-nucleate endosperm; note that the nucleus lying nearest the chalazal end (numbered 16) shows a considerable accumulation of starch grains around it.

formed from its divisions remain within the limits of the sheath which forms a sort of collar around the zygote (Figs. 6, 7). This becomes less sharp, however, at about the octant stage of the embryo, as by this time most of the starch grains disappear. Slightly later only a thin sheath of plasma and nuclei remains around the embryo (Fig. 12b), and subsequently even this remnant of the collar merges with the general endosperm which is still in the free nuclear stage.

Meanwhile, at about the 16-nucleate stage of the endosperm, one nucleus situated towards the chalazal end also gathers some dense cytoplasm and starch grains around it (Fig. 5). Consequently this portion begins to take a deeper stain (Fig. 6) and soon presents the appearance of a coenocytic cyst



FIGS. 6-14. Figs. 6, 7. Stages in the development of the endosperm collar around the zygote ( $\times 450$ ). Fig. 8. Chalazal end of an embryo sac at the 16-nucleate stage of the endosperm, showing one endosperm nucleus surrounded by dense plasma ( $\times 450$ ). Figs. 9-11. Subsequent stages in the development of the basal coenocytic cyst ( $\times 450$ ). Fig. 12. Diagram of longitudinal section of a seed showing the free nuclear endosperm, young embryo, and the basal coenocytic endosperm cyst ( $\times 40$ ). Fig. 12a. Basal coenocytic endosperm cyst from Fig. 12, enlarged ( $\times 450$ ). Fig. 12b. Embryo and surrounding endosperm from Fig. 12. Note the disappearance of the starch grains ( $\times 450$ ). Fig. 13. Diagram of longitudinal section of a seed at the stage when cell formation begins in the endosperm nuclei ( $\times 40$ ). Fig. 14. Diagram of longitudinal section of a seed at a later stage showing endosperm tissue being taken up by the embryo ( $\times 40$ ).

closely packed with starch grains and nuclei (Fig. 10). Later the starch disappears and the cyst shows a pointed basal end penetrating into the chalaza (Figs. 10, 11, 12). Although devoid of any definite separating wall, the upper surface of the cyst seems to become hardened and delimited from the rest of the embryo sac (Figs. 12, 12a).

The remaining nuclei of the endosperm, which occupy the middle region of the embryo sac, continue to divide rapidly and form a peripheral layer which becomes more and more massive and gradually encroaches upon the central cavity. Wall formation commences only at the time of differentiation of the cotyledons and proceeds centripetally. A further increase in the endospermal tissue is brought about by the continued divisions of some of the peripheral endospermal cells, so that in the seed the endospermal tissue occupies the entire space surrounding the embryo and serves as a source of nutriment to the latter.

#### DISCUSSION

Schnarf and Stenar, who have studied the development of the endosperm in *Hypericum*, agree that the early divisions of the primary endosperm nucleus are not followed by wall formation, and it is only later that a multinucleate cyst-like enclosure, resembling a 'Basalapparat' of the type usually seen in the Helobiales, develops at the chalazal end. It seems that Palm was not able to ascertain its exact origin and was therefore misled in assuming that the endosperm was of the Helobiales type. My observations, which agree with those of Stenar (1938), show that the endosperm is nuclear, but in view of the differentiation of a distinctive basal cyst it may perhaps be designated as the *Hypericum* form, for convenience of description. It, of course, still remains to be seen whether a similar mode of development occurs in other members of the Parietales or other angiosperms, and whether the differentiation of the basal cyst can take place at an earlier stage than that seen by Stenar and myself.

#### SUMMARY

In *Hypericum mysorens* Heyne the primary endosperm nucleus divides without wall formation. At about the 8-nucleate stage, one nucleus takes up a position near the zygote, becoming surrounded by dense plasma and starch grains and dividing to form a group of nuclei which forms a prominent mass around the proembryo, but subsequently becoming merged with the general endosperm.

At about the 16-nucleate stage, another nucleus situated towards the chalazal end also gathers round it starch grains and dense cytoplasm and gives rise to a conspicuous coenocytic cyst which later becomes separated from the rest of the endosperm by a limiting membrane. This structure has a distinctive appearance and persists for a long time, finally degenerating as such without merging into the general endosperm.

The development of the endosperm is of the nuclear, not the Helobiales type.

I thank Dr. P. Maheshwari of Dacca University for kindly going through the manuscript and giving valuable suggestions.

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# Inverted Polarity of the Embryo Sac of Angiosperms and its Relation to the Archegonium Theory

BY

B. G. L. SWAMY

(Bangalore, South India)

With six Figures in the Text

RECENTLY Swamy and Thirumalachar (1945) considered the 'inverted polarity', or the 'reversed polarity', of the angiospermous embryo sacs as affording some evidence for the archegonium theory of Porsch (1907). Since this paper was forwarded for publication some of the points raised in it require modification, so an opportunity is here taken to modify viewpoints and to illustrate the situation with reference to the embryo sac of *Crinum asiaticum* L. At present only the structure of the ripe embryo sac of this plant is described, the remaining aspects of its life history being reserved for a later occasion; hence previous work is not reviewed here.

## STRUCTURE OF THE EMBRYO SAC

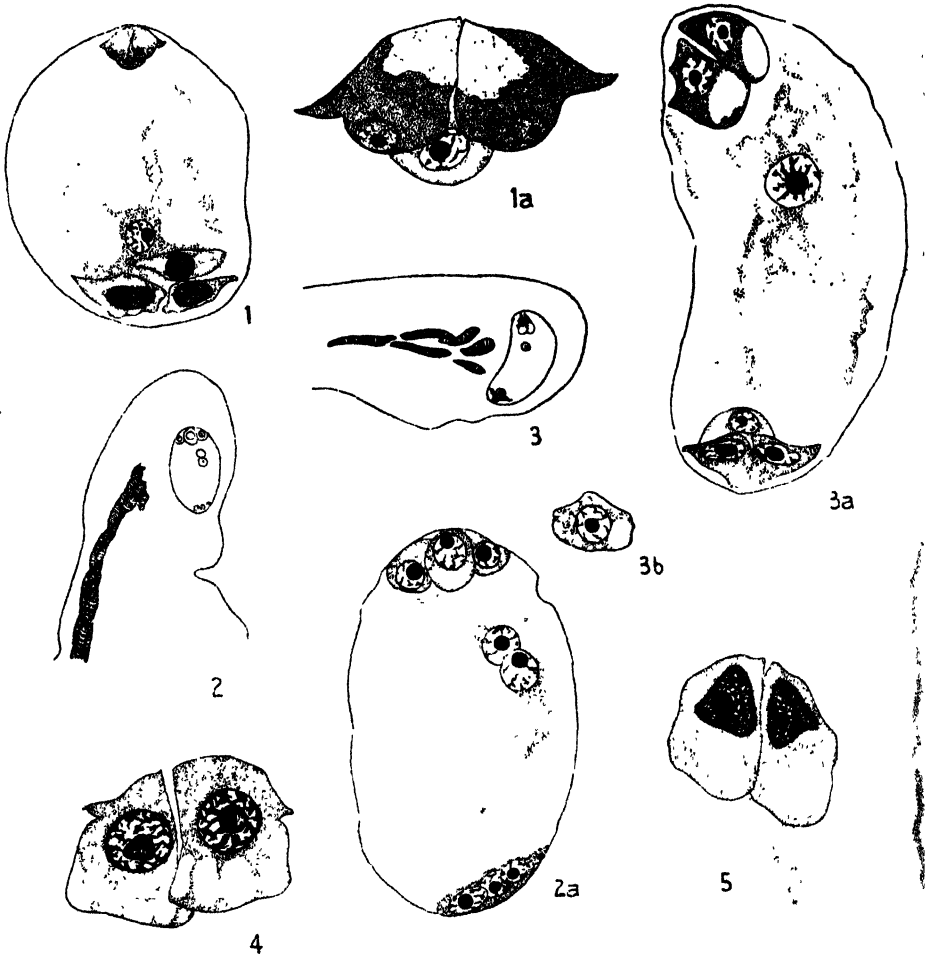
An unorganized 8-nucleate embryo sac of *Crinum asiaticum* may organize in any one of the following methods: (1) The group of 4 nuclei which lies nearer to the micropylar end<sup>1</sup> of the ovule develops into an egg apparatus (2 synergids and 1 egg cell) and a free polar nucleus. The other group of 4 nuclei functions as 3 antipodals and 1 antipodal polar nucleus (Fig. 1). The synergids develop prominent beak-like processes (Fig. 1a) and a non-stainable cyaline apical region. The antipodals always organize into cells and become hypertrophied. The corresponding polar nucleus shows no movement upwards, remaining stationary; the micropylar polar nucleus moves downwards to the antipodal polar nucleus and fusion takes place near the antipodals (Fig. 1). It may be particularly noted that the position of the secondary embryo-sac nucleus is always nearer to the antipodals and not towards the egg apparatus, both in the cases described above and in those described below.

(2) The group of 4 nuclei which lies nearer to the micropylar end of the ovule organizes into 3 antipodal nuclei and a polar nucleus; the other group of 4 nuclei at the opposite end develops into an egg apparatus and the other polar nucleus (Figs. 2 and 2a). Such instances as these were quite frequent, and in all the cases observed the antipodals did not show any cellular organization at this stage; however, they too hypertrophied later. The fusion and

<sup>1</sup> The ovule of *Crinum* is long known to be ategumentary; hence the presence of a micropyle is a matter of doubt. However, this point is receiving study by the present writer. The terms 'micropyle' and 'antipodal' are used here to denote the respective poles as if a micropyle was in evidence.



ultimate position of the polar nuclei took place at this, 'antipodal', end of the sac.



FIGS. 1-5. Fig. 1. Normal, mature embryo sac. Note the position of the secondary embryo-sac nucleus nearer to the antipodals ( $\times 110$ ). Fig. 1a. Egg apparatus from Fig. 1 enlarged ( $\times 240$ ). Fig. 2. An embryo sac in relation to the ovule and placenta, where the polarity of the embryo sac is 'inverted' ( $\times 40$ ). Fig. 2a. Embryo sac enlarged from Fig. 2 ( $\times 110$ ). Fig. 3. An embryo sac in relation to ovule and placenta, where the sac shows the organization of egg apparatus at both poles ( $\times 40$ ). Fig. 3a. Embryo sac enlarged from Fig. 3 ( $\times 110$ ). Fig. 3b. Antipodal egg cell of the embryo sac represented in Fig. 3a (from an adjacent section) ( $\times 110$ ). Figs. 4 and 5. Antipodal synergids ( $\times 240$ ).

(3) In this category may be described those embryo sacs where both groups of 4 nuclei develop an egg apparatus and there is thus one at each end. The synergids of the 'antipodal' end showed great similarity to typical synergids in their shape, vacuolation, and position of the nucleus (Figs. 3, 3a, and 5); they even developed beak-like projections (Figs. 3a and 4). However, the only

difference between the synergids of the fertilisable egg apparatus (developed at the micropylar end) and those of the egg apparatus organized from the antipodal group of 4 nuclei is that the apical, non-stainable, hyaline region of the former is absent in the latter. The egg cell of the 'antipodal' egg apparatus is typically egg-like (Fig. 3*b*).

#### THE EMBRYO SAC IN RELATION TO PORSCH'S THEORY

An admirable critical review of the various phylogenetic views of the embryo sac of angiosperms has been published by Schnarf (1929) and a simple enumeration of some of the important points will suffice for the present purpose.<sup>1</sup> Firstly may be mentioned the views of those authors who recognize the general homology of the embryo sac of angiosperms with the prothallium of gymnosperms, but do not attempt to find homology between the various components of these two gametophytes. This opinion is chiefly sponsored by Coulter and Chamberlain (1903). They write: 'Whether they [the cells of the egg apparatus] represent three archegonia or the egg cell and canal cells of one archegonium seems pressing morphology to absurdity.' They further seem to think it futile to go farther into the question and that there is no sense in trying to investigate the homologies of the component elements of the angiospermous embryo sac. The present writer agrees with Prof. Schnarf that such an attitude is too clearly an abandonment of an attempt to explain the embryo sac of angiosperms.

Next are the views of those who believe that the individual components of the angiospermous embryo sac were all originally potential gametes and that the diversity seen is a secondary character. When we consider the normal type of development of the angiospermous embryo sac, we find that the ontogeny runs according to definite rules: division of the macrospore nucleus into the primary micropylar nucleus and the primary antipodal nucleus, their migration to the respective poles soon after division, their very similar organization into groups of 3 cells and a free nucleus at either end. It may also be noted that it is not the position in which the nuclei accidentally find themselves but their ontogeny that determines their behaviour. These facts relating to the normal type are apart from the original similarity of the various components of the embryo sac. Certain marked instances of the nuclei of the female gametophyte assuming the function of the egg cell, the freedom of certain nuclei in their organization, and some abnormal methods of fertilization have, however, been advocated by the sponsors of this view as providing evidences for their argument. But it will be shown later that these phenomena are to be interpreted in an altogether different light.

Still another school envisages each of the components of the egg apparatus as an entity by itself; the adherents of this school believe that either organs or cells of the egg apparatus find their homologue in the prothallium of gymnosperms. This view was headed by Strasburger and seems to be prevalent

<sup>1</sup> A large portion of some of the following paragraphs are rendered from Prof. Schnarf's original article.

to-day in a large circle. He argued that both in the embryo sacs of angiosperms and gymnosperms naked nuclei are formed in the beginning, but in the angiosperms this condition terminates at an early stage by the formation of 3 cells at each pole. He compared the antipodals and synergids with the prothallium cells and the egg cell with a reduced archegonium. Further, the angiospermic endosperm formation, commencing after fertilization, is assumed to be a continuation of the original interrupted process, which, in angiosperms, requires the stimulus of fertilization. It must be admitted that this view does not explain why the antipodal end should always show a definite plan, with development similar to that of the micropylar end. In short, the 8-nucleate embryo sac of angiosperms corresponds according to Strasburger to an undifferentiated prothallium of the gymnosperm; side by side with the attainment of the angiosperm stage, it has differentiated into its various components—synergids, egg, polars, and antipodals. In other words, embryo-sac formation is a new acquisition and a belated prothallium formation of the gymnosperm is the embryo sac of the angiosperm.

✦ This view has made some progress during subsequent years. Gymnosperms like *Welwitschia* and *Gnetum* and angiosperms like *Peperomia*, *Euphorbia*, &c., were adduced as intermediate stages in the evolution of the angiospermous embryo sac from the gymnosperm prothallium. Some of the botanists who have endeavoured to elaborate this Gnetalean origin of the angiospermous embryo sac are Clakowsky (1901), Lotsy (1899), Karsten (1902, 1918), Pearson and Thomson (1916), &c.<sup>1</sup> They derive the 16-nucleate embryo sacs of angiosperms from the prothallium of *Gnetum*, in which every nucleus is considered to be a potential gamete, through the curtailment of the progress of divisions from the mother cell till the development is perfected, and this is accompanied by differentiation and specialization. Such an interpretation should explain why, in the case of angiosperms in general, 5 nuclei (2 synergids and 3 antipodals) have given up their character as gametes and have become cells with other functions. Pearson and Thomson (1916) give an explanation for this, resting on novel assumptions: that the absence of polarity in *Gnetum*, the presence of which is a very marked character of the angiospermous embryo sac, is the result of the innumerable number of nuclei crowded in the small space of the prothallium of *Gnetum*; that double fertilization is a monstrosity. This Gnetalean school of thought suffers chiefly because of the presumptions which the adherents had to make that cell formation at the micropylar and chalazal poles and the differentiation of the components of the embryo sac of angiosperms are a secondary characteristic, which appears only at the angiosperm stage. As has already been pointed out, the present state of our knowledge does not offer any basis for the presumption that the components of the egg apparatus or of the embryo sac are, in general, phylogenetically equivalent with one another. Further, the 16-nucleate embryo sacs of angiosperms cannot be brought forward as evidence for this view,

<sup>1</sup> Recently Fagerlind (1942) also supports this view. See also literature cited by Thompson (1916).

because they must only be considered as derived types as they develop from a 'coeno-macrospore'.

The several hypotheses which consider the egg apparatus, and partly also other nuclei of the embryo sac, as homologous with an archegonium make no attempt to explain the formation of the ripe embryo sac. It is evident that in this structure there is to be found a fertile region containing the egg cell and a chalazal sterile region corresponding with a vegetative prothallial region; why the antipodals, either always or at a certain stage of development, appear in threes is not explained in any of these views. Ward (1880) finds in the micropylar group of 4 nuclei of the normal type of the angiospermous embryo sac a formation homologous with an archegonium; he explains that the embryo sac is made up of two prothallial structures. One of them produces a rudimentary archegonium (egg cell and synergids) and one vegetative nucleus (upper polar nucleus); the lower spore produces four vegetative cells (antipodals and antipodal polar nucleus). Evidently this means that Ward was unable to explain the antipodal cells. Further, his view is entirely untenable because it rests on the assumption that two macrospores come together for the formation of the embryo sac.

Schurhoff (1919, 1926) in more recent years has made an attempt to homologize the egg apparatus of angiosperms as consisting of two archegonia. According to him, the egg cell and one synergid (the latter representing one ventral canal nucleus) constitute the first archegonium; the other synergid and upper polar nucleus constitute the second. He considers the antipodals as delimited prothallial cells. The examples chosen by him are all those standing outside the typical behaviour of angiosperms. Many of these instances have in recent times been shown to tend towards a normal behaviour. The data available overwhelmingly show that the egg cell and a synergid on the one hand and the second synergid and the upper polar nucleus on the other hand are not sister cells. It must be concluded that Schurhoff's views are untenable.

In 1907 Porsch propounded his archegonium theory of the derivation of the angiospermous embryo sac. The normal type is regarded by him as being the most primitive. Each group of 4 nuclei at the micropylar and antipodal ends of the embryo sac represents an archegonium, consisting of 2 neck cells, 1 egg cell, and 1 ventral canal nucleus. The egg apparatus with the upper polar nucleus represents the micropylar archegonium, and the antipodals and the lower polar nucleus the antipodal archegonium. The egg cell corresponds to the egg cell, the synergids to the neck cells, and the upper polar nucleus to the ventral canal nucleus of the upper archegonium. In the antipodal complex, one of the antipodals—more frequently the middle one—corresponds to the egg cell, the remaining two to the neck cells, and the lower polar nucleus to the ventral canal cell of the lower archegonium.

According to Porsch, side by side with the tendency towards the reduction of the prothallium are associated the following features: (1) a tendency among angiosperms to concentrate the archegonia, which amongst the most primitive forms lie scattered, and to form an archegonial complex, (2) a tendency to

reduce the number of archegonia whereby the individual archegonia lose the reproductive capacity and serve the purpose of feeding the fertile one, (3) correlated with the second tendency is the further reduction in the number of nuclei constituting the antipodal complex. The end result of these tendencies is the normal type of embryo sac of angiosperms, where we find the formation of only naked cells. Thus, according to Porsch, the vegetative portion of the prothallia of the Pteridophyta and gymnosperms has completely disappeared in the angiosperms, whereas, peculiarly enough, in the archegonium the same essential features are still maintained—ventral canal nucleus, neck cells, and egg cell.

Porsch's theory, as may be seen, tries to explain adequately in logical sequence all the structures of the embryo sac and the theory is supported by the great authority of Schnarf. Embryological researches of late have contributed considerably towards substantiating this theory. Still, however, some criticisms are raised against Porsch's views and some significant objections and answers to them may be summarized as follows:

(1) Magnus (1913) raises the objection, a difficulty felt by many others, that the chain of connecting links in the disappearance of prothallium from the gymnosperms to the angiosperms is not to be seen in living forms. This is not a serious objection in the opinion of Schnarf, who cites the instance of the male gametophyte of angiosperms, and considers a parallel phenomenon to have taken place in the history of the female gametophyte. He further points out that in gymnosperms the macrospore nucleus passes through a number of divisions, and a few nuclei that arise as a consequence become archegonium initials; but that in the angiosperms, on the other hand, the macrospore nucleus divides only once and each of the resulting nuclei becomes the initial of an archegonium.

(2) It is well known that in gymnosperms the archegonium originates by two nuclear divisions which are followed by immediate wall formation. Brown (1908) objects to the archegonium theory because in the angiosperms wall formation generally takes place after the 4 nuclei are formed. Porsch himself feels a consideration of this objection to be superfluous as he considers the formation of naked nuclei—or in other words, the suppression of cell-wall formation—as a strongly marked developmental tendency amongst gymnosperms and that in the highest ranks this tendency comes clearly into expression. Further, Schnarf has attempted to show the trivial nature of the objection by considering the conditions prior to cell-wall formation in a cell containing a large amount of sap, and above all fast-growing, as is the case in the young embryo sac, which condition is not present in the archegonium initial which lies in the midst of prothallial tissue in most of the gymnosperms.

(3) Ernst (1908) contends that in most cases the analogy between the egg apparatus and the antipodal group fails; especially, the startling process of multiplication of the antipodal cells finds a simple explanation in the earlier interpretation of the antipodals as vegetative prothallial cells. Against this criticism, Schnarf argues that the multiplication, and, also the occasional

hypertrophied condition of the antipodals, is without doubt a secondary phenomenon, which arises independently among the different embryo-sac types. It should be noted in this connexion that whatever may be the ultimate number of antipodals, it does pass through a 3-cell stage during ontogeny.

(4) One objection which is worthy of consideration is the applicability of Porsch's theory to the Adoxa type of embryo sacs. This objection is raised and answered by Prof. Schnarf himself. In this type all the four macrospore nuclei contribute to the mature embryo sac. If each of these gives rise to a quadruple group (one archegonium), then it would be similar to the macrospore of *Oenothera*. On the other hand, in the Adoxa type of embryo sac where only two quadruple groups originate, each of the macrospore nuclei divides only once and the daughter nuclei of the two pairs form one archegonium. Schnarf thinks that this difficulty is solved if we interpret the Adoxa type as a very highly derived formation, in which the play of reduction in the number of divisions is seen to reach a climax.<sup>1</sup>

(5) Maheshwari (1937) asks: 'what reason is there for the ventral canal nucleus (upper polar nucleus) to leave its position above the egg and come down into the centre of the embryo sac to fuse with another nucleus from the chalazal end which is also the ventral canal nucleus of a second archegonium?' The polar nuclei of the embryo sac of angiosperms are comparable, according to Porsch, to the ventral canal nuclei of the two archegonia. As Schnarf has observed, the ontogeny of the egg apparatus corresponds with the theory in so far as the egg and the polar nucleus are sister nuclei. Porsch himself has cited certain gymnospermous archegonia where the ventral canal nucleus occupies a lateral position near the egg nucleus or a position even below it. To presume, to overcome this difficulty, that an exchange of position has taken place during phylogeny of the archegonium appears unnecessary to Schnarf. We may consider here another possible explanation. Whatever may be the cytological nature of the endosperm, haploid, diploid, triploid, or polyploid, and whatever may be the morphology of this tissue (a secondary embryo modified for the purpose of serving as nutrition to the sexually produced embryo, a belated prothallial tissue, a tropophyte, or a xeniophyte), its fundamental function remains the same, and this is being nutritive. We have ample evidence for the view that the fusion of two or more nuclei and its subsequent divisions results in a polyploidal tissue with richer nutritive value and of prolonged persistence. Strasburger long ago expressed the opinion that the polar nuclei fuse because they are in some way senile and so require a stimulus to ensure further development. In the majority of angiosperms this stimulus results from the intervention of the second male nucleus during double fertilization. Thus we may suggest: (i) that a nutritive tissue is essential for the normal development of the sexually produced embryo; (ii) that the polar nuclei are responsible for building up such a tissue either (a) in extremely rare cases by their own division without previous fusion, a phenomenon

<sup>1</sup> In the *Plumbago* type also we find each macrospore nucleus dividing only once into an egg cell and a corresponding polar nucleus.

which indicates their individual power to divide, or (b) with a previous fusion, which indicates a slight senility which is overcome by mutual fusion; or (c) by a division only after triple fusion, which indicates their greater senility and the necessity of the intervention of the second male nucleus. (iii) It has been supposed for a long time that synergids produce some substance which attracts the pollen tube. It appears likely that some similar stimulant is responsible for the mutual attraction of the two similar nuclei as well as for the attraction of the fused polar nucleus (secondary embryo-sac nucleus) towards the egg apparatus.

We may now recall what has been said earlier, that the micropylar and antipodal ends of the normal type of embryo sacs of angiosperms show great similarity during ontogeny. There are many instances on record where the mature organization of the antipodal complex resembles that of the upper half of the embryo sac. Firstly, there are examples where either one, two, or all the three antipodal cells show a resemblance either to the synergids or to a typical egg cell, so far as their shape, vacuolation, disposition of nucleus, &c., are concerned. Such instances are too numerous to be listed. It may further be noted that in *Crinum* the antipodals often develop 'hook-like' processes which are occasionally met with in normal synergids. Next are those examples where the antipodal complex assumes the character of the egg apparatus whereas the micropylar group organize themselves either as antipodals or again as an egg apparatus. Such instances have come to be described as cases of 'inverted polarity' or 'reversed polarity', and are listed below:

Plant.	Author.	Remarks.
1. <i>Allium nigrum</i>	Modilewski, 1931	Even the 'filiform apparatus' is seen in the egg apparatus organized at the antipodal end.
2. <i>A. odorum</i>	Tretjakow, 1895	
	Haberlandt, 1922, a and b	
	Modilewski, 1925, 1931	
3. <i>A. paniculatum</i>	Modilewski, 1931	
4. <i>A. paradoxicum</i>	Weber, 1929	The author reports the fertilization of the antipodal egg by a male nucleus.
5. <i>Aster Novae-Angliae</i>	Chamberlain, 1895	
	Goldflus, 1898	
	Operman, 1904	
6. <i>A. undulatus</i>	Operman, 1904	
7. <i>Atamasco texana</i>	Pace, 1913	
8. <i>Cistus laurifolius</i>	Chiarugi, 1925	
9. <i>Eriodendron anfruticosum</i>	Thirumalachar and Khan, 1941	
10. <i>Fuchsia marinka</i>	Tackholm, 1915	
11. <i>Heptapleurum venulosum</i>	Gopinath, 1943	
12. <i>Karthalsella Dacrydi</i>	Rutishauser, 1935	
13. <i>Lindelfolia longiflora</i>	Svensson, 1925	
14. <i>Nothoscordon fragrans</i>	Stenar, 1932	
15. <i>Oenothera gigas</i>	Beth, 1938	
16. <i>O. lamackiana</i>	" "	

Plant.	Author.	Remarks.
17. <i>Paris quadrifolia</i>	Ernst, 1902	
18. <i>Rhapalocnemis phalloides</i>	Lotsy, 1901	
19. <i>Rudbeckia bicolor</i>	Maheshwari and Srinivasan, 1943	
20. <i>Saccharum</i> sp.	Dutt and Subba Rao, 1933	
21. <i>Trillium grandiflorum</i>	Ernst, 1902	
22. <i>Ulmus americana</i>	Shuttuck, 1905	
23. <i>U. hollandica belgica</i>	Leliveld, 1935	
24. <i>Woodfordia floribunda</i>	Joshi and Venkateswaralu, 1935	

Many have taken advantage of such examples of the antipodals, where one, two, or all show marked similarity to one or two of the components of the egg apparatus, to subscribe to the view that the various components of the embryo sac are potentially alike.<sup>1</sup> That this view cannot be accepted has been shown on a previous page. And the extreme cases of this phenomenon classified as 'inverted polarity' or 'reversed polarity' have been looked upon as chance anomalies in organization and no attempt has been made till now to explain their significance.

*Laphamos* sp. and *Cotylanthera tenuis* (an apomictic plant) in addition to 'inverted polarity' show some other interesting features which mask the correct interpretation. Here the ovules are reported to be devoid of integuments, and the egg apparatus is organized towards the funiculus and the antipodals at the opposite end. According to Oehler (1927), these two associated features when taken together show that the ovules are externally orthotropic, i.e. the embryo sac is oriented as in the seeds of anatropous ovules.

In addition to *Cotylanthera*, *Atamasco texana* (Pace, 1913) is again an apomictic plant, but at the same time exhibits 'inverted polarity' in as high as 50 per cent. of the ovules. This has induced certain authors to relate such peculiar organization of the embryo sac to the apomictic habit of the plant. The fact that *Crinum* shows 'inverted polarity' of the embryo sac in approximately 30 per cent. of the ovules and yet exhibits a normal sexual method of reproduction with double fertilization not only makes the above contention doubtful but also renders the phenomenon of 'inverted polarity' significant. The following points contained in or deduced from Porsch's theory become important for the present purpose: (1) The polar similarity between the upper and lower halves of the embryo sacs of angiosperms, which appears in the perfected condition and more distinctly in the development, is an appearance that is understandable only if homologous organs develop at both polar ends. (2) The micropylar archegonium has maintained its sexual function while the lower one has undergone secondary modifications to serve the purpose of nutrition in various ways; multiplication, hypertrophy, &c.; early disappearance of the antipodals, or even a curtailment of division of the primary antipodal nucleus, come into this category. (3) The normal type of embryo sac of angiosperms is to be regarded, with Schnarf and others, as the most primitive because in this type is to be found many of the primitive characters

<sup>1</sup> See Joshi (1939).



in their clearest expression, including the tendency towards the secondary modifications of the antipodal complex. (4) We can also see from the data that these general tendencies and features are maintained even in the rest of the derived types—*Allium*, *Peperomia*, *Fritillaria*, *Adoxa*, &c. (see Fig. 6). More especially the formation of the quadruple groups of nuclei and their resemblance to the egg apparatus is shown clearly in *Penaea*, in various species

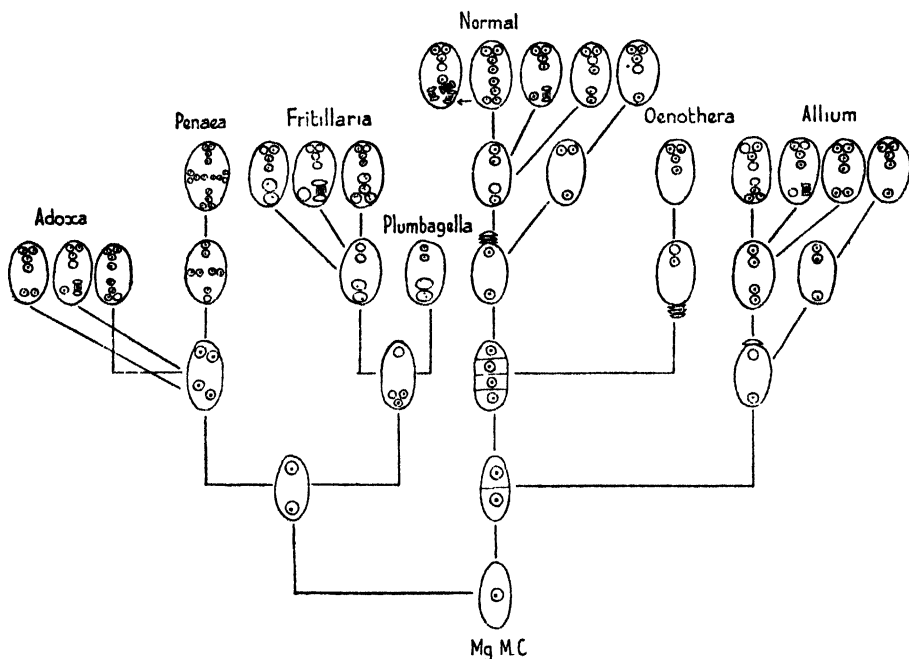


FIG. 6. Diagram of the derivation from the fundamental normal type of the various types of embryo sacs of angiosperms; the various secondary modifications of the antipodal half of the embryo sac are seen independently in almost every type. The horizontal and vertical lines in the diagram point to the direct derivation of the respective type, and the slanting lines to the secondary modification of the antipodal archegonium. Several forms of the 16-nucleate embryo sacs under the *Peperomia* type, other than the *Penaea*, are considered to be derived still further and hence are not represented in the diagram.

of *Peperomia*, and even in the much derived type, *Fritillaria*.<sup>1</sup> This feature is also maintained with great tenacity in the ontogeny and also in the ripe condition of the *Adoxa* type of embryo sac.<sup>2</sup>

These considerations lead the author to suggest the following interpretation of all the cases of what has hitherto been described as 'inverted polarity' or 'reversed polarity' of the embryo sac. It is true that such instances are sporadic; but though sporadic they are well distributed and cannot be without significance. Such cases, as well as those that develop an egg apparatus at

<sup>1</sup> In a very recent paper Maheshwari and Srinivasan (1944) have reported instances of the mature embryo sac showing an antipodal complex bearing striking similarities to the normal egg apparatus organized at the micropylar end of the same embryo sac.

<sup>2</sup> See Zweifel's paper on *Balanophora* (1939).

both poles, lend considerable support to the archegonium theory of Porsch, in that they represent instances of 'hypothetical' intergrades where the antipodal archegonium also expresses itself in the fullest degree. In other words, cases of 'inverted polarity' or 'reversed polarity' are nothing but the suppression of the micropylar archegonium; the antipodal archegonium has attained to fertility whereas the micropylar archegonium has suffered a secondary modification. Those cases of embryo sacs which develop an egg apparatus at each end also indicate the homology of the antipodals—that they belong to a reduced or modified archegonium of the gymnosperm. That usually no fertilization of the egg of the antipodal archegonium has been observed<sup>1</sup> cannot be taken as a serious objection, for the egg in question is far away from the point of entry of the pollen tube.

#### SUMMARY

The development of the structures at the two poles of the embryo sac of *Crinum asiaticum* L. shows great similarity, so much so that about 30 per cent. of the ovules at the time of fertilization show a similar organization of egg apparatus at each end; in addition to this, cases also occur frequently where the antipodal group of nuclei organize themselves as egg apparatus, while the micropylar group differentiates as antipodals.

Various views regarding the phylogeny of the embryo sac of angiosperms are briefly stated. And the view expressed by Porsch and Schnarf that each quadruple group of nuclei in the normal type of embryo sac of angiosperms represents an archegonium of the gymnospermous type is held to be the most tenable hypothesis. Instances of embryo sacs hitherto described as having 'inverted polarity' or 'reversed polarity', and also instances of embryo sacs that develop an egg apparatus at both ends (like the embryo sac of *Crinum*), are interpreted as having considerable significance in the light of Porsch's theory in that the antipodal quadruple group is also capable of exhibiting itself as a typical egg apparatus.

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<sup>1</sup> Fertilization of the antipodal egg has been shown or assumed in *Aster* by Operman (1904), and *Ulmus* by Shuttuck (1905).

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# Growth and Fruiting of certain Ascomycetous Fungi as influenced by the Nature and Concentration of Carbohydrate in the Medium

BY

L. E. HAWKER

AND

S. D. CHAUDHURI

(Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London)

With one Figure in the Text

BROWN (1925) reported that glucose in increased concentrations was more effective than starch in producing mycelial growth of strains of *Fusarium fructigenum*. He pointed out that the whole of the starch in the medium might be consumed and yet the weight of mycelium produced was less than that on a medium containing a similar amount of glucose and suggested that differences in respiration rate might account for these results. Conidial production tended to increase with increasing starch concentration but was depressed by increase in that of glucose. Sucrose and maltose gave results similar to those with glucose.

Certain carbohydrates may be arranged in a series according to their effects on growth and formation of perithecia by *Melanospora destruens* (Hawker, 1939). Mycelial growth increased with increasing concentration of glucose or fructose up to 10 per cent., but showed a slight falling off at 20 per cent. Perithecia were most numerous at a relatively low concentration (0.5 per cent. in the presence of a standard dose of growth substances). The number fell off rapidly as the concentration of these sugars increased. Growth on a sucrose medium was, at all concentrations, less than that on a medium containing a similar amount of glucose, but perithecia, which were very few with low concentrations of sucrose, reached a maximum at a relatively high concentration (10 per cent.). Maltose, lactose, starch, and arabinose were intermediate in effect between glucose and sucrose. The effects of glucose and sucrose were further investigated and it was concluded that 'the superior value, for sporulation, of high concentration of sucrose lies in part in the maintenance, through enzymatic inversion of a fairly uniform low concentration of hexose sugars'.

The present paper describes the effects of concentrations of glucose, fructose, sucrose, maltose, starch, and lactose on growth and fruiting of *Podospora* sp., *Sordaria fimicola*, *Chaetomium cochloides*, *Melanospora zamiae*, *Ceratostomella adiposa*, and *Pyronema confluens*.

*Experimental methods* were in general similar to those described in previous papers. The fungi were grown on agar media in Petri dishes of 9 cm. diameter and incubated at 25° C. unless otherwise stated. Replication in some experiments was less than in earlier work owing to the necessity of conserving materials. Growth substances were supplied in the form of liquid medium A (Asthana and Hawker, 1936) in which *Rhizopus suinus* had grown for 2 weeks at laboratory temperature. This 'staled' medium contained no glucose but the amounts of the other original ingredients remaining in the medium were not estimated. The filtered medium received the ingredients of agar medium A, with the exception of glucose, together with amounts of the various carbohydrates to give concentrations of the latter of 0.5, 1.0, 2.0, 5.0, and 10.0 per cent. and was made up to its original volume with distilled water and autoclaved. Occasionally an extract of lentils was used as an alternative source of growth substances (Hawker, 1936). Fruiting was estimated by the method of Asthana and Hawker (1936).

*Growth and fruiting of Podospora sp.*

*Glucose.* Effects were essentially similar to those obtained by Hawker (1939) for *M. destruens*. Mycelial growth increased and the hyphae became dark coloured with increase in glucose. Fruiting fell off rapidly with increase in concentration above the optimum. This optimum was slightly higher with *Podospora* than with *M. destruens*.

*Fructose.* Results were similar in type to those with glucose, but fructose was inferior as a source of carbon for fruiting (i.e. perithecial frequency was lower on any given concentration of fructose than on a similar one of glucose) in contrast to results with *Melanospora* where glucose and fructose produced almost identical effects.

*Sucrose.* Mycelial growth and fruiting were poor on the lowest concentration (0.5 per cent.) used. Both increased with increasing concentration up to the highest (10.0 per cent.) used, but the number of perithecia formed was small and growth was thin and colourless. Thus sucrose was less suitable for *Podospora* than for *Melanospora*.

*Maltose and starch.* Mycelial growth increased with increase in either maltose or starch. At all concentrations growth was better than on similar concentrations of sucrose but inferior to that on glucose. Fruiting reached an optimum at a concentration of 2.0 per cent. of either starch or maltose (cf. 1.0 per cent. for glucose) above which it fell off more gradually than with glucose.

*Lactose.* Mycelial growth increased with increase in concentration of lactose but was less vigorous than with glucose. Fruiting increased up to the limit of the experiment at level (10.0 per cent.) when perithecial frequency was the highest recorded for this fungus (viz. 12.7). The perithecia were smaller than those on media containing glucose, fructose, sucrose, starch or maltose.

*Growth and fruiting of Sordaria fimicola*

*Glucose.* Results were essentially similar to those with *M. destruens* and *Podospora* sp.

*Fructose.* Results are not available owing to loss of fertility in culture.

*Sucrose.* Response was similar to that of *Podospora*.

*Maltose.* Results are not available.

**Starch.** Mycelial growth continued to increase up to the highest amount tried (10.0 per cent.). Perithecial frequency was highest (18.3) at a concentration of 2.0 per cent. above which it fell off more slowly than with glucose.

**Lactose.** Effects were intermediate between those of glucose and starch. The optimal concentration for fruiting (1.0 per cent.) gave a relatively high perithecial frequency (viz. 10.0).

#### *Growth and fruiting of Chaetomium cochloides*

**Glucose.** Mycelial growth increased with concentration up to the maximum tried. Fruiting increased up to a concentration of 2.0 per cent. above which it fell rapidly.

**Fructose.** Results similar to those with glucose, but the number of perithecia at any concentration was slightly less than with glucose.

**Sucrose.** Results approached those with glucose, but the actual number of perithecia formed at the optimum concentration was greater.

**Maltose.** Mycelial growth increased with increase in concentration up to the highest tried (5.0 per cent.), but fruiting reached a maximum at 2.0 per cent. The number of perithecia formed at this optimal concentration was comparable with that on sucrose, but fruiting fell off more slowly above this value than with sucrose.

**Starch.** Both growth and fruiting increased with concentration up to the maximum (10.0 per cent.) tried and the actual number of perithecia formed was large.

**Lactose.** Results were similar to those with starch, but the actual numbers of perithecia were higher at every concentration, while individual perithecia were small.

#### *Growth and fruiting of Melanospora zamiae*

**Glucose.** Aerial mycelium increased with increase of glucose concentration, but the colonies did not reach the edge of the plates and had an irregular wavy margin of the type which Brown (1925) observed with *F. fructigenum* and associated with 'staling'. There were a few perithecia at the lowest concentration. At higher concentrations no mature fruit bodies were formed and the number of immature ones fell off rapidly with increasing concentration.

**Fructose.** Mycelial growth was much less than on glucose. The diameter of the colonies decreased with increase in sugar, but the proportion of aerial growth increased. Perithecia were most numerous at concentrations of 0.5-1.0 per cent., above which they decreased and none were formed at 5.0 per cent.

**Sucrose.** Growth was thin and spreading on the lower concentrations, but was thicker and there was a tendency to develop a wavy margin on the higher ones. Perithecia were most numerous on the lower concentrations, but the number fell off more slowly with increasing concentration than with glucose or fructose.

**Maltose.** Growth was thin and spreading at all concentrations. The number of perithecia increased slightly with concentration.

**Starch.** Growth was thin and spreading at all concentrations. The number of perithecia fell off slowly with increase in concentration.

**Lactose.** Similar to maltose but perithecia were smaller.

#### *Growth and fruiting of Ceratostomella adiposa*

**Glucose.** Mycelial growth was good and increased with concentration up to 2.0 per cent., but at 5.0 per cent. no white aerial mycelium was formed and the margin



of the colony was irregular, indicating staling. Perithecia were most numerous at the lowest concentration.

*Fructose.* Growth and fruiting resembled that on glucose, but perithecia were more numerous at all concentrations than on the corresponding glucose media.

*Sucrose.* The reduction in growth at 5.0 per cent. sucrose was much less than at 5.0 per cent. glucose. Perithecia were fewer at low concentration than with similar concentrations of glucose or fructose. At higher concentrations they were intermediate in number between those on glucose and fructose.

*Starch, maltose, and lactose.* Growth was thin and spreading (starvation type) at all concentrations. The number of perithecia increased slightly with increase in concentration of lactose and fell off slightly with increase in that of maltose and more rapidly with that of starch. Perithecia formed on media containing lactose were smaller than on the other carbohydrates.

Conidia were formed on all media.

#### *Growth and fruiting of Pyronema confluens*

Since light is necessary for the production of apothecia by this fungus the cultures were grown at laboratory temperature.

*Glucose.* Mycelial growth was good and increased with concentration until at 10.0 per cent. there was a dense mass of white mycelium. At low concentrations few apothecia were formed, but fruiting was good at 5.0 per cent., after which it fell off rapidly until at 10.0 per cent. only a few immature apothecia were formed.

*Fructose.* Results were similar to those with glucose, but fruiting was depressed at a lower concentration than with glucose.

*Sucrose.* The results closely resembled those with glucose.

*Maltose.* Growth increased with increasing concentration within the limits of the experiment, but fruiting fell off above 5.0 per cent.

*Starch.* Results with starch resembled those with glucose and sucrose, but the number of apothecia fell off more slowly above the optimal concentration of starch (viz. 5.0 per cent.) so that they were still numerous at 10.0 per cent.

*Lactose.* Growth was of a starvation type, increasing slightly with increased concentration. Apothecia were never numerous, but the number increased with the concentration of lactose.

These results are brought together in Table I and are summarized in Table II which gives the percentage concentration of each carbohydrate optimal for fruiting of the six fungi here studied and also of *M. destruens* (Hawker, 1939).

It is clear that these fungi are essentially similar in their reactions to glucose and fructose. Growth and fruiting increased with increase in hexose sugars up to a concentration, varying with the species, above which fruiting fell off rapidly while mycelial growth continued to increase. In the previous paper (Hawker, 1939) it was shown that with further increase in sugar concentration mycelial growth of *M. destruens* also fell, and it was concluded that this was due to osmotic effects. Such a response is represented by curves  $F_1$  and  $M_1$  of the figure. Relatively high concentrations of hexose sugars thus encourage the development of vegetative hyphae but depress and finally inhibit fruiting.

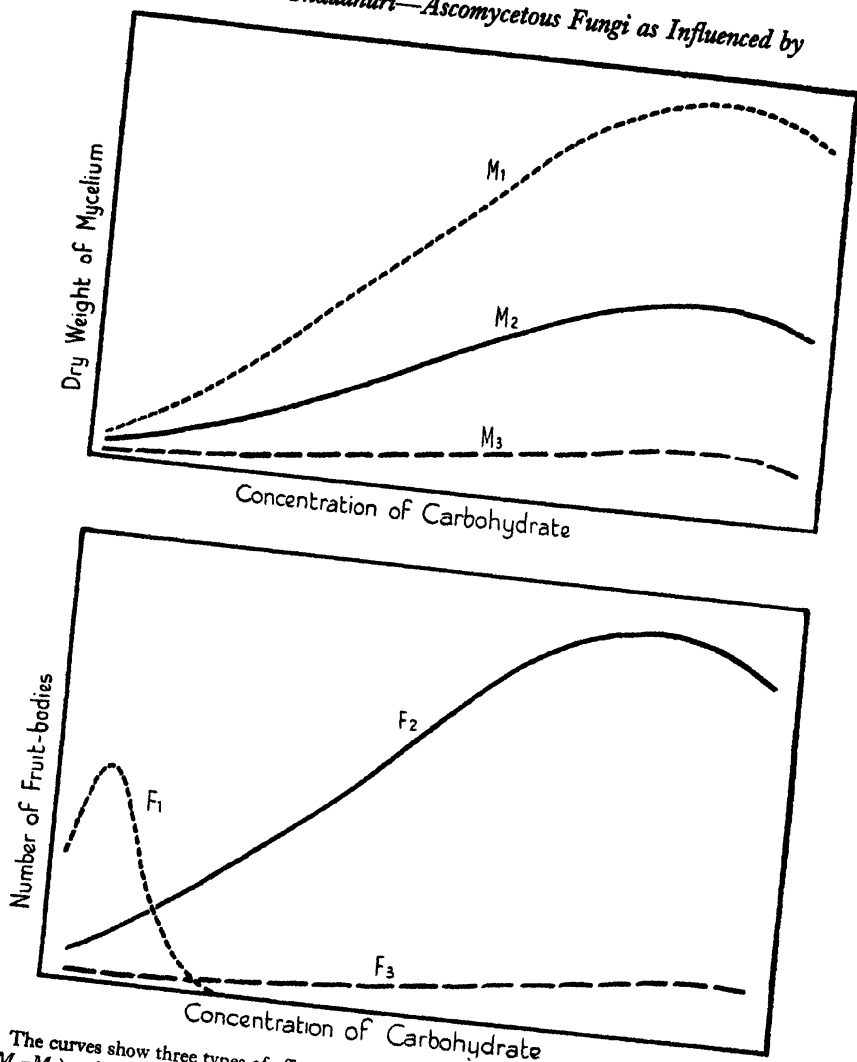
TABLE I  
Fruiting of Fungi on various Carbohydrates

Fungus.	Carbohydrate.	Percentage concentration of carbohydrate.				
		0.5	1.0	2.0	5.0	10.0
<i>Podospora</i> sp.	Glucose	4.6	4.7	2.8	Few	0
	Fructose	1.7	3.9	2.0	Few	0
	Sucrose	0.8	1.1	2.0	2.8	4.5
	Maltose	2.4	4.2	5.1	3.9	3.1
	Starch	3.0	5.2	6.5	4.6	Few
	Lactose	4.3	8.9	10.0	11.5	12.7
<i>Sordaria fimicola</i>	Glucose	8.2	10.2	Few	0	0
	Sucrose	0.7	0.9	1.3	3.6	4.6
	Starch	7.2	12.5	18.3	9.2	6.2
	Lactose	6.2	10.0	7.8	Few	Few
<i>Chaetomium cochloides</i>	Glucose	4.3	5.6	6.3	Few	0
	Fructose	4.0	5.3	6.0	Few	0
	Sucrose	3.9	4.7	8.3	Few	Few
	Maltose	4.0	4.9	8.6	6.3	4.2
	Starch	3.1	4.1	6.1	12.1	12.4
	Lactose	5.7	6.4	8.4	12.0	13.8
<i>Melanospora zamiae</i>	Glucose	0.4	0.1	0	0	—
	Fructose	3.8	4.0	2.0	0	—
	Sucrose	3.1	2.4	2.0	0	—
	Maltose	2.3	2.4	2.5	2.8	—
	Starch	2.4	2.4	2.0	1.9	—
	Lactose	2.0	2.2	2.4	2.8	—
<i>Ceratostomella adiposa</i>	Glucose	4.5	1.2	0.6	0.2	—
	Fructose	4.6	2.2	1.9	1.2	—
	Sucrose	2.3	2.2	1.4	0.6	—
	Maltose	4.0	4.0	2.7	2.6	—
	Starch	3.1	2.1	1.2	0.6	—
	Lactose	2.2	2.4	2.5	2.6	—
<i>Pyronema confluens</i>	Glucose	2.9	3.6	4.9	8.7	Few
	Fructose	2.5	3.2	5.1	6.3	Few
	Sucrose	2.4	3.1	4.2	8.4	Few
	Maltose	3.0	3.9	4.8	7.0	Few
	Starch	2.6	3.7	6.6	9.0	9.6
	Lactose	Few	Few	0.9	1.2	1.4

The frequency of mature fruit bodies was estimated by the method of Asthana and Hawker (1936).

TABLE II  
Percentage Concentration of Carbohydrate optimal for Fruiting

	Glucose.	Fructose.	Sucrose.	Maltose.	Starch.	Lactose.
<i>M. destruens</i> . . .	0.5	0.5	5.0	0.5	1.0-2.0	0.5
<i>Podospora</i> sp. . .	1.0	1.0	10.0+	2.0	2.0	10.0+
<i>S. fimicola</i> . . .	1.0	—	10.0+	—	2.0	1.0
<i>C. cochloides</i> . . .	2.0	2.0	2.0	2.0	10.0+	10.0+
<i>M. zamiae</i> . . .	0.5	1.0	0.5	5.0+	0.5-1.0	5.0+
<i>C. adiposa</i> . . .	0.5	0.5	0.5	0.5-1.0	0.5	5.0+
<i>P. confluens</i> . . .	5.0	2.0-5.0	5.0	1.0	10.0+	10.0+



The curves show three types of effect of concentration of carbohydrate on mycelial growth ( $M_1$ - $M_3$ ) and on fruiting ( $F_1$ - $F_3$ ).  $M_1$  and  $F_1$  illustrate typical response to hexose sugars (e.g. *M. destruens* on glucose).  $M_2$  and  $F_2$  represent the effect of a more complex carbohydrate on a fungus which can break it down at a moderate rate (e.g. *M. destruens* on sucrose).  $M_3$  and  $F_3$  show the extreme type where the ability of a fungus to break down a complex carbohydrate is so poor that a starvation type of growth results (e.g. *P. confluens* on lactose).

The response of these fungi to the more complex carbohydrates showed more variation. Thus, at one extreme, *Pyronema* responded similarly to glucose and sucrose. The optimum value (5.0 per cent.) with both sugars was higher than with other fungi on glucose, but small-scale experiments with concentrations intermediate between 5.0 and 10.0 per cent. showed that fruit-

ing fell off rapidly with increase in sugar concentration above 5.0 per cent. and thus the response is essentially of the  $F_1$  type of the figure for both glucose and sucrose. It may be suggested that this fungus, like *F. fructigenum* described by Brown (1925), inverts sucrose rapidly so that a sucrose medium approaches a glucose one in effect.

At the opposite extreme, represented by *Pyronema* with lactose and *Podospora* and *Sordaria* with sucrose, both growth and fruiting continued to increase gradually with increase in sugar concentration but neither reached a high value within the limits of the experiment. This type of response, represented by curves  $F_3$  and  $M_3$  of the figure, may be explained by a failure to produce an adequate amount of the appropriate enzyme so that a starvation type of growth results.

Between these extremes are all gradations of response. Thus *M. destruens* showed a starvation type of growth on low concentrations of sucrose only less severe than that shown by *Podospora* or *Sordaria*, but growth and fruiting increased with sugar concentrations until, at 10.0 per cent., growth was good (although less than on 10.0 per cent. glucose) and perithecia were numerous (curves  $M_2$  and  $F_2$  of the figure). It might be expected that *M. destruens* inverts sucrose more rapidly than do *Podospora* and *Sordaria* but much less rapidly than does *Pyronema*. Starch and lactose, however, are presumably broken down more rapidly than sucrose by *M. destruens* since the optimal concentrations are between those of sucrose and glucose. Maltose is probably broken down more rapidly than either starch, lactose, or sucrose since the optimal value is the same as that for glucose, although the fact that a maltose medium is not thereby rendered identical with a glucose one is shown by the more gradual falling off of fruiting above the optimal concentration.

Responses similar to that of *M. destruens* to starch are shown by *Podospora* and *Sordaria* to starch and by *Chaetomium* to maltose. The response of *M. destruens* to sucrose resembles that of *Podospora* to lactose, of *Chaetomium* to starch and lactose, and of *Pyronema* to starch, while the response of *Chaetomium* to sucrose resembles that of *Melanospora* to maltose.

Thus it is likely that these fungi differ in their ability to produce various enzymes and consequently in their power to utilize more complex carbohydrates.

That the rate at which more complex substances are broken down to hexose sugars does not entirely account for the observed differences in response was pointed out by Hawker (1939) for *M. destruens* since the effect of sucrose could not be reproduced exactly by the artificial maintenance of a concentration of hexose sugar equal to that occurring in the sucrose medium. The fact that the actual numbers of fruit bodies produced by a particular fungus at the optimal concentration of various carbohydrates differed considerably (e.g. perithecial frequencies of 4.7, 3.9, 5.1, 6.5, and 12.7 at the optimal concentration of glucose, fructose, maltose, starch, and lactose respectively were obtained with *Podospora*) also indicates that factors other than rate of enzyme production are involved. Nevertheless a comparative study of the relation between

rate of enzyme production and the type of response to these carbohydrates would be of interest. Unfortunately, owing to war conditions, it was not possible to carry out detailed analyses of this kind, but a further comparison of the responses of *Podospora*, *Chaetomium*, and *Pyronema* to sucrose was undertaken.

*Comparison of response of Podospora, Chaetomium, and Pyronema to sucrose*

These three fungi were grown at laboratory temperature in liquid medium *A* in which glucose was replaced by 2.0 per cent. sucrose plus the standard dose of growth substances. Samples were taken 4, 7, and 11 days after inoculation and the total residual sugar and reducing sugar in the media and the dry weight of mycelium were determined. The results are given in Table III.

TABLE III

	Days after inoculation.	Percentage total sugar in medium.	Percentage reducing sugar.	Dry wt. of mycelium (mg./100 cc. medium).	Sugar (mg.) consumed per mg. dry wt. of mycelium.
<i>Podospora</i>	4	1.86	0.05	45	3.1
	7	1.74	0.06	75	3.6
	11	1.67	0.03	115	2.9
<i>Chaetomium</i>	4	1.93	0.04	42	1.7
	7	1.40	0.30	325	1.8
	11	0.66	0.77	780	1.7
<i>Pyronema</i>	4	0.69	0.63	600	2.2
	7	0.03	Trace	880	2.2
	11	Trace	None	900	2.2

After 4 days, growth of *Podospora* and *Chaetomium* was slight and a negligible amount of sugar had been used while growth and consumption of sugar by *Pyronema* were already considerable. Seven days after inoculation *Pyronema* still showed the greatest growth and consumption of sugar, with *Chaetomium* intermediate between *Pyronema* and *Podospora*. Four days later *Pyronema* had used practically all the sugar and *Chaetomium* more than half, while *Podospora* had used very little and made comparatively poor growth. The amount of reducing sugar in the medium of the *Podospora* cultures was always small, while in the *Chaetomium* cultures it was small at first but increased with age of the cultures. In the *Pyronema* cultures the amount of reducing sugar was high after 4 days but fell in older cultures owing to the rapid using up of the sugar by this fungus. Similar experiments gave comparable results.

These results were in agreement with the suggestion that the different responses of these three fungi to sucrose were, in part at least, due to the rate at which the sugar was inverted. Thus the starvation growth of *Podospora* is related to a very low concentration of reducing sugar in the medium and the similarity in response of *Pyronema* to glucose and sucrose is correlated with the production of a relatively large amount of reducing sugar in the sucrose

medium. Thus it was concluded that invertase was produced most rapidly by *Pyronema* and most slowly by *Podospira*. To test this hypothesis, mycelia of these three fungi were grown on medium *A* (with 2.0 per cent. sucrose) plus 0.2 per cent. lentil extract. The mycelia were washed, dried in a desiccator and equal weights of the dried mycelia were ground up with a standard quantity of silver sand. Equal weights of the resulting powders were added to a standard 0.1 per cent. sucrose solution. The percentages of reducing sugar present after one hour in the solutions to which the dried and macerated mycelia of *Pyronema*, *Chaetomium*, and *Podospira* were added were 0.10, 0.08, and 0.03 respectively. Thus differences in the response of these fungi to sucrose can be correlated with their ability to produce invertase.

It has already been pointed out that rate of enzyme production cannot account for all the differences in response of these fungi to various carbohydrates. It is clear, however, that, other things being equal, fruiting of these organisms is best on a medium with a relatively high initial concentration of a di- or polysaccharide which the fungus breaks down to hexose sugars neither so quickly as to approach the effect of a high initial concentration of glucose nor so slowly that a starvation type of growth results, but at such a speed that there is a moderate concentration (about 0.5 per cent. for *M. destruens* but higher with some other species) of hexose sugars in the medium for a relatively long period. Mycelial growth, however, is always most vigorous on media with relatively high initial concentrations of hexose sugars or of more complex carbohydrates when these are broken down so rapidly as to be indistinguishable from glucose in effect.

#### SUMMARY

The effects of a range of concentrations of glucose, fructose, sucrose, maltose, starch, and lactose on growth and fruiting of seven ascomycetous fungi were examined. Response to glucose or fructose was always of the same type, viz. mycelial growth increased with increase in sugar up to a relatively high concentration, while fruiting reached a maximum at a low concentration (which varied with the species), above which it fell off rapidly. Response to the more complex carbohydrates was of three types: (1) similar to that to hexose sugars, (2) a starvation type of growth at low concentrations and a slight increase in both growth and fruiting with increase in concentration, (3) an intermediate type where growth and fruiting were poor at low concentrations but increased with increase in amount of carbohydrate until both were good.

The type of response of three fungi to sucrose was correlated with the rate at which the sugar was inverted and with the amount of invertase produced per unit dry weight of mycelium.

It is suggested that these differences in response can be partly, but not entirely, explained by differences in the rate at which a particular fungus can break down complex carbohydrates to hexose.

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# Growth Inhibiting Action of Urine Extract on Seedlings

BY

E. DOROTHY BRAIN

THE inhibiting effects of extracts of urine, and of auxin, hetero-auxin, and other substances have been described for the growth of seedlings when applied apically to the stem. The H 11 extract used in the following series of experiments is an alcoholic extract of urine prepared by Hosa Research Laboratories, and used in treatment of tumour growth. It is known to have an inhibiting effect on the root growth in *Avena*. One of the inhibitory substances present in the extract is  $\beta$ -indole-acetic acid and an addition has been made of its sodium salt (Thompson, 1943). Fractionation of the extract to isolate its active principles has yielded evidence that a substance, or substances, similar to the quinone series is present (Thompson, 1944). Seedlings of *Vicia Faba* and *Pisium sativum* (var. Stratagem) have been treated with H 11 extract, and fractions of H 11 and its effects on the growth of lateral buds examined. Experiments have also been performed using pure  $\beta$ -indole-acetic acid and its sodium salt and three members of the quinone series.

## METHOD

Plants were grown in pots of soil in the light until three to four internodes high and then placed in the dark for 24 hours before experiment and for 48 hours after treatment. The treated plants were kept under glass covers in a moist atmosphere, and the maximum and minimum temperature recorded daily.

Stems were decapitated in two ways: (1) at the tip, removing the tip and terminal leaf, termed 'cut above growth', (2) at the node below which growth has ceased, removing all the actively growing internodes, termed 'cut below growth'. The cut surface of the stem was dried with filter-paper and then covered with a lanoline paste containing the substance to be tested. The concentration of the paste used was equivalent to 3.5 c.c. H 11 in 6 gm. lanoline diluted with lanoline to 1: 2 and 1: 20. Paste was also prepared by emulsifying 3.5 c.c. H 11 solution with 3.5 c.c. liquid paraffin and mixing it with 2 gm. of lanoline. Controls were treated with distilled water in lanoline or paraffin and lanoline. The inhibiting effect is calculated as the percentage difference between the averages for the total length of side shoots in treated and control plants. The lengths of side shoots were measured with a mm. rule and added for each plant and the average for the totals of each series of plants estimated. Any increase in the height of the cut stems was recorded and the width of the cut stems was also noted. An approximate measure of the swelling of stems



was arrived at by calculating the percentage increase in width for each plant and the average for a series, but it is realized that this was not an exact method. The results recorded are only of qualitative value.

#### RESULTS FOR H 11 PASTE

I. *Vicia Faba*. Epicotyls 'cut above growth'. Treatment with H 11 paste at the top of the cut stems caused swelling and growth of callus at the cut surface, similar to the effects of  $\beta$ -indole-acetic acid described by Laibach and Fischnich (1935). Lateral buds were slightly slower than controls in developing and side shoots were inhibited 68.9 per cent. Similar effects were produced by treatment with the paste diluted 1:2 and the side shoots were inhibited 51.6 per cent. 1:2 paste applied along one side of the stems caused marked curvatures, the treated sides becoming convex. Development of the buds was definitely delayed and the growth of side shoots was inhibited 83 per cent.

II. *Pisum sativum*. Epicotyls 'cut below growth'. When treated with paste in three different concentrations no increase in the height of the cut stems was noted nor did swelling of the stems occur except slightly in a few isolated cases. No difference was noted in the order in which the buds developed, the second bud from the base of the stem usually starting growth first. The buds were slower developing after treatment with the strong paste and their growth was markedly inhibited. Paste applied to the side of the stems inhibited the growth of side shoots and caused curvatures in the stems.

Epicotyls 'cut above growth'. Treatment of plants at the top of the cut stems caused increased growth in the cut stems and marked swelling. The amount of swelling varied with individual plants, but the average percentage increase in the width of the stem for the strong paste was 150 per cent. and 70 per cent. for the paste diluted 1:2 and 1:20. The growth of the side shoots was inhibited.

Some typical series of results are given in Table I.

#### EXPERIMENTS WITH DIFFERENT FRACTIONS OF H 11 EXTRACT

H 11 extract was believed to contain a growth inhibitor other than the  $\beta$ -indole-acetic acid and its sodium salt, and three fractions of the extract were prepared by the Hosa Research Laboratories to separate the active constituents. The method used for their preparation was as follows:

(1) Excess copper sulphate was added to neutral H 11 and the resultant green precipitate filtered off and retained. Hydrogen sulphide was bubbled through the filtrate to remove excess copper and the gas expelled by boiling on a water bath for a few minutes. After evaporation on a water bath it was brought to pH 7 and made up to its original volume. This preparation was labelled 80 K.

(2) The green precipitate was dissolved in dilute HCl and the soluble fraction filtered off leaving a black tar, which was retained. The soluble fraction was bubbled through with hydrogen sulphide and after boiling brought to pH 7 and made up to 100 c.c. This was labelled 80 L.

(3) The black tar was next dissolved in 5N. NaOH, H<sub>2</sub>S bubbled through, brought to pH 7 and made up to 100 c.c. and labelled 80 M.

Experiments were performed with *Vicia Faba* and *Pisum sativum* seedlings, 'cut above growth'. The paste I applied was prepared by emulsifying 3.5 c.c. of each solution with 3.5 c.c. liquid paraffin and mixing with 2 gm. of lanoline. A more concentrated paste II was prepared by using 5 c.c. of each solution with 3.5 c.c. paraffin and 2 gm. lanoline. In *Vicia Faba* application of paste I,

TABLE I  
*Inhibition of Side Shoots with H II Paste*

<i>Vicia Faba</i> . Epicotyls 'cut above growth'					
Application.	No. used.	No. growing side shoots.	Average length of side shoots per plant (cm.)	Percentage inhibition.	Time in days.
1:0 top	6	5	3.86 ± 1.283	68.9	10
1:2 top	7	7	6.0 ± 1.852	51.6	10
1:2 side	8	8	2.1 ± 0.455	83.02	10
Control	10	10	12.4 ± 1.865	—	10

<i>Pisum sativum</i> . Epicotyls 'cut below growth'					
Application.	No. used.	No. growing side shoots.	Average length of side shoots per plant in cm.	Percentage inhibition.	Time in days.
1:0 top	10	6	1.2 ± 0.575	81.6	10
Control	4	4	6.52 ± 0.768	—	10
1:2 top	10	7	2.4 ± 0.680	55.4	10
Control	5	5	4.44 ± 0.919	—	10
1:20 top	10	10	4.65 ± 0.761	37.0	9
Control	6	6	7.38 ± 1.238	—	9
1:0 side	15	7	1.4 ± 0.428	78.6	10
1:2 side	9	5	1.9 ± 0.841	71.0	10
Control	5	5	6.45 ± 0.596	—	10

<i>Pisum sativum</i> . 'cut above growth'					
Application.	No. used.	No. growing side shoots.	Average length of side shoots per plant in cm.	Percentage inhibition.	Time in days.
1:0 top	5	4	1.04 ± 0.38	70.2	7
1:2 top	5	4	1.44 ± 0.575	55.7	7
Control	9	9	3.5 ± 0.537	—	7
1:20 top	15	10	3.13 ± 0.568	29.4	7
Control	10	10	4.43 ± 0.285	—	7

of the three fractions, to the top of cut epicotyls resulted in slight swelling of the stems in each series. Inhibition of the side shoots was noted in plants treated with 80 K, and to a less extent with 80 L, but those treated with 80 M showed an increase in the growth of side shoots.

In *Pisum sativum* the results for paste I were variable for 80 K and 80 L, but 80 M showed inhibition of growth of side shoots. Treatment with paste II resulted in inhibition of side shoots for 80 K and 80 M, but 80 L had no inhibiting effect. The average increase in width of stems for plants treated with 80 K was 50 per cent. An increase in width of more than 100 per cent.

was noted after treatment with 80 L and 80 M. It seems evident from these results that 80 K contains an inhibitory substance, distinct from the alkali soluble fraction, 80 M, which has been found by Thompson et al. (1943) to be stimulatory to tumour growth.

Table II summarizes the results for two typical series of experiments.

TABLE II  
*Experiments with three Fractions of H 11*

<i>Vicia Faba</i> epicotyls					
Paste I.	No. used.	No. growing side shoots.	Average length (cm.) of side shoots per plant.	Percentage inhibition.	Days.
80 K	5	4	3.98 $\pm$ 1.069	52.2	12
80 L	5	5	6.66 $\pm$ 0.7204	20.0	12
80 M	5	5	9.06 $\pm$ 2.012	none	12
Control	4	4	8.325 $\pm$ 0.7662	+9.15	12
<i>Pisum sativum</i> epicotyls					
Paste II.	No. used.	No. growing side shoots.	Average length (cm.) of side shoots per plant.	Percentage inhibition.	Days.
80 K	4	3	3.2 $\pm$ 2.792	42.86	10
80 L	3	3	5.73 $\pm$ 0.9935	none	10
80 M	4	3	3.25 $\pm$ 1.119	+2.3	10
Control	5	5	5.6 $\pm$ 0.721	41.67	10

#### EXPERIMENTS WITH $\beta$ -INDOLE-ACETIC ACID AND SODIUM INDOLE-ACETATE

As it was known that one of the inhibitory substances in H 11 extract was  $\beta$ -indole-acetic acid and that the addition of its sodium salt had been made in the preparation of the extract, it seemed desirable to compare results for H 11 with the effects of similar treatment with  $\beta$ -indole-acetic acid. Thimann and Skoog (1934) compared the inhibition caused by different concentrations of hormone preparations on *Pisum*, and found complete inhibition of the lowest bud after treatment of the cut stem with heteroauxin in a concentration of 7,000 units per c.c. For concentrations of 1,000–5,000 units per c.c. they recorded an inhibition of 60 per cent. for the total bud growth. They found no swelling or increase in height of the cut stems as a result of treatment. The results recorded in Table III for *Pisum* show inhibition of 69.6 per cent. after treatment with  $\beta$ -indole-acetic acid of a concentration of approximately 6,250 units per gramme in lanoline, and 76.8 per cent. increase in the width of the cut stems but no appreciable increase in their height.

A comparison of results for treatment with  $\beta$ -indole-acetic acid and a solution neutralized with sodium hydroxide shows more swelling in stems treated with the sodium salt but no evidence of difference in the growth of side shoots (see Table IV). It is interesting to note that Avery (1937) has recorded the fact that with the *Avena* test the potassium salt of  $\beta$ -indole-acetic acid is twice as active as  $\beta$ -indole-acetic acid.

TABLE III  
*Experiments with  $\beta$ -indole-acetic Acid*

<i>Pisum sativum</i> , 'cut above growth'						
Application.	No. used.	No. growing side shoots.	Average growth (cm.) of side shoots per plant.	Percentage inhibition.	Percentage increase in width.	Average increase (mm.) in height.
2,063 units per gm.	12	9	1.17 $\pm$ 0.2034	53.0	43.6	4.25 $\pm$ 1.217
6,250 units per gm.	12	6	0.775 $\pm$ 0.307	69.6	76.8	5.6 $\pm$ 0.844
Control . . . . .	9	9	2.55 $\pm$ 0.524	—	3.0	3.4 $\pm$ 0.631

TABLE IV  
*Pisum sativum epicotyls treated with  $\beta$ -indole-acetic Acid and Na indole-acetate*

Concentration applied.	Percentage increase in width.			Average growth of side shoots (cm.).			Number used.		Days.
	$\beta$ -indole-acetic acid.	Na indole-acetate.		$\beta$ -indole-acetic acid.	Na indole-acetate.		$\beta$ -indole-acetic acid.	Na indole-acetate.	
138 units per gm. lanoline.									
'Cut above growth'	27.0	37.6		3.75 $\pm$ 1.185	4.7 $\pm$ 0.759		7	9	8
'Cut below growth'	7.0	—		2.45 $\pm$ 0.614	4.95 $\pm$ 0.424		10	8	8
416 units per gm. lanoline.									
'Cut above growth'	33.0	84.6		2.44 $\pm$ 1.272	1.75 $\pm$ 0.591		6	6	8
833 units per c.c. agar.									
'Cut above growth'	95.0	130.0		4.0 $\pm$ 0.351	0.93 $\pm$ 0.933		3	3	10
'Cut below growth'	11.0	35.3		3.1 $\pm$ 0.883	1.26 $\pm$ 0.819		3	3	10
'Cut below growth'	47.3	56.8		1.3 $\pm$ 0.415	1.1 $\pm$ 0.352		10	10	7

The solutions were applied in lanoline and 3 per cent. agar. Concentrations are expressed as the approximate number of units of growth substance per gramme lanoline and per cubic centimetre of agar. 1 mg. of  $\beta$ -indole-acetic acid being taken as equal to 250,000 units.

#### INHIBITORY EFFECT OF ANTHRAQUINONE

The analysis of H 11 extract has yielded evidence that as well as  $\beta$ -indole-acetic acid and its sodium salt another inhibitory substance is present similar to a derivative of the quinone series (Thompson, 1944).

TABLE V

*Pisum Epicotyls, 'cut above growth', treated with Anthraquinone Derivatives*

Application.	Average increase in height of stem (mm.).	Average growth of side shoots per plant (cm.).	Percentage inhibition.	No. of plants.	Days.
Conc. 1:1.5					
Anthraquinone saturated solution.	5.8 $\pm$ 1.175	0.4 $\pm$ 0.187	73.4	5	5
Anthraquinone 2:6 disulphonic acid refined sodium salt 0.1%	5.6 $\pm$ 1.631	0.7 $\pm$ 0.338	54.4	5	5
1:5 di-hydroxy- anthraquinone 0.1%	1.4 $\pm$ 0.5099	1.98 $\pm$ 0.848	none +35.7	5	5
Control	3.66 $\pm$ 1.253	1.48 $\pm$ 0.377	—	6	5
Conc. 1:1					
Anthraquinone saturated solution	3.5 $\pm$ 1.957	0.825 $\pm$ 0.370	33.0	4	5
Anthraquinone 2:6 disulphonic acid refined sodium salt 0.1%	6.75 $\pm$ 1.931	0.45 $\pm$ 0.333	64.0	4	5
1:5 di-hydroxy- anthraquinone 0.1%	2.0 $\pm$ 0.333	2.2 $\pm$ 0.400	none +78.0	3	5
Control	3.0 $\pm$ 1.732	1.23 $\pm$ 0.815	—	3	5

It has also been found that pure substances in the anthraquinone series have an inhibitory effect on the growth of malignant cells (Thompson, 1944). It therefore seemed of interest to test similar substances on seedlings, and the following solutions in water were used: (1) a saturated solution of anthraquinone, (2) anthraquinone 2:6 di-sulphonic acid refined sodium salt, 0.1 per cent., (3) 1:5 di-hydroxy-anthraquinone 0.1 per cent. Experiments were carried out as before and the solutions applied in lanoline paste in a concentration of approximately 1:1.5 and 1:1.

The results summarized in Table V show slight increase in the height of cut stems and inhibition of growth of side shoots after treatment with anthraquinone and anthraquinone di-sulphonic acid sodium salt. Plants treated with 1:5 di-hydroxy-anthraquinone show less growth of the cut stems than the

controls and increased growth of side shoots. No swelling of cut stems occurred.

### DISCUSSION

The fact that inhibition of lateral buds may be produced by the application of auxin to the apical cut surface of decapitated stems has led to the theory that inhibition is correlated with the auxin supply in the main axis. According to Snow (1939) the auxin descending in the main stem reacts or co-operates with another factor to produce the inhibitory effect on the growth of lateral buds. Other workers consider the development of the buds to be dependent on the auxin itself reaching the buds in the optimum concentration for their development (Thimann and Skoog, 1934, and van Overbeek, 1938). Borgström (1939) explains the association of the swelling in the stem with bud inhibition as being both indications of a transverse stream of auxin from the phloem, induced by the decapitation of the main stem. In nature, lateral buds fail to grow out because they are short of hormones or auxin. He regards experimental retardation of buds as true inhibition when, through a concentrated supply of auxin, the buds grow radially instead of elongating, a secondary result of the transverse supply of auxin being swelling in the main stem. Borgström finds support for this theory in the work of van Overbeek, who demonstrated different gradients in intact, decapitated, and auxin-treated seedlings.

The experiments described above give support to Borgström's theory of the swelling since they show that inhibition occurs quite independently of the swelling of the stems. When stems of *Pisum* were 'cut below growth' the inhibition was sometimes greater than when the growing part was treated and swelling occurred; and it has been found that anthraquinone acts as an inhibitor without causing swelling.

The fact that substances other than auxin can cause bud inhibition indicates that it may not be simply an auxin effect. Powell (1944) using anthraquinone and other substances has explained their inhibitory action on malignant cells as being due to their property of tanning proteins and stabilizing the discrete fibrils of the malignant cells and rendering them less able to reproduce themselves. The possibility that a similar activity may be associated with inhibition in plant cells justifies the assumption that inhibitive factors other than auxins may be involved.

### SUMMARY

1. Experiments have been performed on cut epicotyls of *Pisum sativum* and *Vicia Faba* to test the action of urine extract (H 11),  $\beta$ -indole-acetic acid, sodium indole-acetate and certain anthraquinone derivatives on the growth of lateral buds.

2. The urine extract caused marked inhibition of the growth of side shoots and swelling in the cut stems.

3. Treatment with  $\beta$ -indole-acetic acid caused inhibition of the growth of side shoots and swelling in the cut stems, but a concentration of 6,250 units per gm. caused less swelling than H 11 extract.

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4. A comparison between the effect of  $\beta$ -indole-acetic acid and sodium indole-acetate showed more swelling in the cut stems after treatment with the sodium salt than with the acid.

5. Solutions of anthraquinone, and the refined sodium salt of anthraquinone di-sulphonic acid caused inhibition of side shoots and slight increase in the height of the cut stems. 1:5 di-hydroxy-anthraquinone showed less growth in the cut stems and increased growth of side shoots, in comparison with control plants.

6. The results indicate that the swelling of the stem which follows application of concentrated solutions of  $\beta$ -indole-acetic acid is independent of the inhibition effect on lateral buds as stated by Borgström (1939); also that other substances, such as members of the quinone series, can inhibit the growth of side shoots.

I am indebted to Mr. J. H. Thompson, Director of Research of Hosa Research Laboratories, for providing the H 11 extracts, and other substances.

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# **The Salt Relations of Plant Tissues**

## **III. Further Observations on the Absorption of Manganese Chloride by Storage Tissue**

BY

WALTER STILES

AND

K. W. DENT

With five Figures in the Text

### INTRODUCTION

**P**REVIOUS papers in this series (Stiles and Skelding, 1940, 1940a) have presented the results of a primary survey of the absorption of potassium and manganese salts by a storage tissue, carrot root. Similar work which was in progress on the absorption of a number of other salts and which had to be suspended on account of the war will, it is hoped, be described at an early date.

In the present paper we give the results of a more extensive study of the absorption of manganese chloride presented to various tissues in a low concentration. The experiments herein described provide further data on the course of absorption of ions by storage tissue and confirm earlier observations and conclusions, some of which had been called in question by Steward and Berry (1943), and also extend the information on the absorption of manganese chloride given in the second paper of this series.

### EXPERIMENTAL METHODS

The general arrangement of the experiments was the same as that used in earlier studies. A number of storage tissues were used, namely roots of carrot (Sutton's Favourite), mangold (Yellow Globe and Red Intermediate), swede (Eclipse), and red beet (Sutton's Crimson Globe). The carrot and beet were grown in the grounds of the University of Birmingham, the swedes were obtained from Harper Adams Agricultural College, and the mangolds from Mr. Cope, of Goldstone, Shropshire. The tissue was used in the form of discs which when cut were 2 cm. in diameter and 1 mm. in thickness. These were washed for various times in aerated running tap-water before transference to solutions of 0.001 M manganese chloride. As before, 20 discs were placed in 200 ml. of solution contained in stoppered bottles of about 420-ml. capacity provided with a hole 1 cm. in diameter in the stopper and shaken continuously and regularly in a water-bath maintained at 25° C. by means of a Sun-vic control. The experiments were generally conducted in replicate.



Stiles and Skelding (1940, 1944) have already dealt with the criticism that this simple arrangement does not provide adequate aeration for the tissue.

In previous work the absorption of both manganese and chloride was followed by estimating the concentration of these in samples withdrawn from the external solutions at various times, a quantity of tissue being withdrawn at the same time in order to preserve the same proportion of tissue to solution throughout the whole course of the experiment. The manganese and chloride were for the most part determined polarographically. Both the use of the polarograph and the estimation of external solutions have been previously criticized by Steward and Berry. Although Stiles and Skelding appear to have been the first workers in this country to use the polarograph for biological work, the instrument had previously been used by continental botanists (e.g. by Prát, 1926; Laine, 1934) and its use has since been much developed in America. Its reliability and convenience should now require no defence. We have, nevertheless, checked its use in our work with other methods. As an alternative method for the estimation of manganese we have used the periodate method, the estimations being made by means of the Spekker absorptiometer. As an alternative method for the determination of chloride titration against silver nitrate was employed, dichlorofluorescein being used as adsorption indicator. Both these methods have the disadvantage, as compared with the use of the polarograph, that much larger samples are needed for a determination. Thus with the polarograph we used 5 ml. of external solution for the determination of manganese and usually only 2 ml. for the determination of chloride, though even smaller samples could have been used, whereas for the estimation of manganese by the periodate method we used 20 ml. of solution and for the estimation of chloride with the use of the adsorption indicator we required 140 to 170 ml. of solution.

As regards the question of analysing the external solution or the tissue, as has been previously pointed out (Stiles and Skelding, 1944), the conditions of the experiment may make it necessary to use one or the other and the investigator may have no choice. But where he has the choice he should unhesitatingly use the external solution. This usually contains negligible quantities of impurities, whereas in the tissue itself the element to be determined is usually accompanied by a much larger amount of other material. This generally necessitates a number of operations in making the desired estimation, the general effect of which is to add greatly to the labour involved and to lower the accuracy of the determination. Where possible the tissue should first be subjected to wet or dry ashing; the use of expressed sap should, in our opinion, be avoided if possible, for the composition of expressed juice is a function of the conditions employed to express it (cf. e.g. Bennet-Clark and Bexon, 1940), and it is difficult to keep these constant. We have, however, made determinations of chloride in the tissue for comparison with determinations made on the external solution. To do this we incinerated the tissue at a low temperature to avoid volatilization of chloride, extracted the resulting ash with water

and determined the chloride by titration with silver nitrate, using dichloro-fluorescein as adsorption indicator.

In the two experiments now to be described discs of carrot-root tissue (Sutton's Favourite) were washed in aerated running tap-water for 23 hours. Six replicates, each comprising 20 discs of tissue in 200 ml. of 0.001 M solution of manganese chloride, were used for each experiment. In the first experiment, after absorption had proceeded for 53.2 hours the manganese and chloride in the solutions of two of the replicates were determined polarographically and chemically as described above. The same determinations were made with two other replicates after 144 hours and with the last two after 264 hours. The results of this experiment are shown in Table I.

TABLE I

*Comparison of Determinations of Manganese and Chloride by Polarographic and Chemical Methods*

Hours.	Manganese absorbed (%).		Chloride absorbed (%).	
	Polarograph.	KIO <sub>4</sub> .	Polarograph.	Adsorption indicator.
53.2	43.7	43.1	0.1	73.5
144.0	52.4	53.8	30.7	28.3
264.0	56.8	58.9	71.1	60.9

The second experiment was similar to the first, but in this the chloride in the discs was determined as well as that remaining in the external solution. The results are shown in Table II.

TABLE II

*Comparison of Determinations of Absorption of Manganese and Chloride by Polarographic and Chemical Methods*

Hours.	Manganese absorbed (%).		Chloride absorbed (%).		
	Polarograph.	KIO <sub>4</sub> .	Polarograph.	Adsorption indicator from external solution.	from tissue.
51.7	39.0	40.2	19.1	11.2	14.4
144.2	48.3	50.2	35.3	30.9	36.6
264.3	50.6	51.7	59.7	55.9	49.8

In a third experiment mangold (Yellow Globe) was used. The conditions of this experiment were similar to those with carrot apart from the fact that

TABLE III

*Comparison of Determinations of Absorption by Mangold Tissue of Manganese and Chloride by Polarographic and Chemical Methods*

Hours.	Manganese absorbed (%).		Chloride absorbed (%).		
	Polarograph.	KIO <sub>4</sub> .	Polarograph.	Adsorption indicator from external solution.	from tissue.
47.7	26.4	28.3	11.5	12.7	14.2
143.8	53.6	56.9	59.8	62.7	60.1
264.0	81.0	84.2	83.2	83.65	84.8

12 samples of tissue were used instead of 6. The results are shown in Table III.

Having regard to the quantities and concentrations measured, the agreement between the different methods of estimation can be regarded as satisfactory, the quantities of manganese and chloride actually present in the samples used for polarographic determinations being less than 0.2 and 0.15 mg. respectively.

#### THE COURSE OF ABSORPTION OF THE IONS OF MANGANESE CHLORIDE

In the previous paper in this series it was shown that the absorption by carrot tissue of manganese from solutions of manganese salts follows a different course from that of the anion (chloride, sulphate, or nitrate). The intake of manganese showed a two-phase course involving an initial phase of rapid absorption as if towards a position of equilibrium, followed by a second phase characterized by long-continued absorption at a slower rate than the initially rapid rate of the first phase. With the anion, on the contrary, no two-phase course was observable, the rate of absorption at first being much slower than that of the cation but later tending to exceed it, so that the disparity between the total amount of the two ions absorbed lessens with time.

These results showed differences from those obtained with potassium salts (Stiles and Skelding, 1940), but were in general agreement with those described by Steward and Harrison (1939) for the absorption of rubidium bromide by potato, in which there was an initially rapid absorption of rubidium accompanied by little or no absorption of bromide, followed by a long-continued absorption of both ions at about the same rate. Two points of difference appeared, however, between the absorption of manganese salts by carrot as described by Stiles and Skelding and that of rubidium bromide by potato as described by Steward and Harrison. As regards the first of these, Stiles and Skelding found that there was generally a definite falling off in the rate of absorption of manganese towards the end of the first phase and before the marked onset of the second phase, whereas, according to Steward and Harrison, the initial phase of absorption of rubidium passes straight over into the second phase without any suggestion of an approach to an equilibrium, and Steward and Berry later (1943) attributed the similar temporary decline in absorption found by Stiles and Skelding for potassium salts to chance variations in the determinations, although they produced no evidence for this assumption. The second point of difference appears in the relative rates of absorption of cation and anion during the second phase. Stiles and Skelding found that although with manganese salts the cation is first absorbed at a much more rapid rate than the anion, during the second phase the anion is absorbed somewhat more rapidly than the cation, so that the difference between the total absorption of the two ions lessens with time. With rubidium bromide, on the other hand, Steward and Harrison reported that after the preliminary period of high cation absorption and low anion absorption the two ions are absorbed at approximately the same rate, so that the difference

between the total amount of the two ions absorbed during the first phase remains constant.

Whatever may be the state of affairs in the absorption of rubidium bromide by potato tissue, there is no doubt that the conclusions previously drawn with regard to the absorption of manganese salts were correct. The experiments described in the previous section of this paper show that with both carrot and mangold tissue approximate equality of absorption of both ions of manganese chloride is finally reached. The temporary slowing down of absorption of manganese by carrot while the first phase passes over to the second was

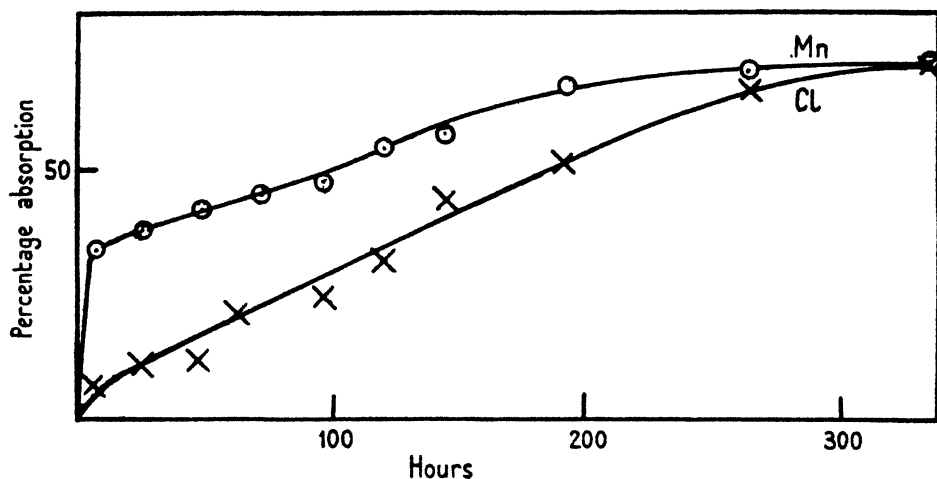


FIG. 1. The course of absorption by carrot-root tissue of the ions of manganese chloride from a solution of an initial concentration of 0.001 M.

abundantly shown in the previous paper in this series; in the further experiments recorded in this section it is shown that the course of absorption by other storage tissues is similar. But, as will be shown in the next section of this paper, the time at which the rate of absorption in the second phase becomes significant can vary, and when this begins so early that it overlaps the first phase the temporary decline in absorption rate may not occur.

#### Carrot.

The discs were cut from carrots (Sutton's Favourite) taken from store on March 9 and washed in aerated running tap-water for 91 hours before transference to 0.001 M manganese chloride. The course of absorption was followed by polarographic determination of manganese and chloride in samples withdrawn at intervals from the solution external to the tissue. The results are given in Table IV and shown graphically in Fig. 1. The gradual increase in excess of anion absorption over that of the cation during the second phase, leading finally to equality in the total absorption of both ions, is shown in the last column of the table.

TABLE IV  
*Course of Absorption of the Ions of Manganese Chloride by Carrot*

Hours.	Absorption of Mn (% of original quantity in external solution).	Absorption of Cl (% of original quantity in external solution).	Mn—Cl.
5.9	33.3	7.4	25.9
24.0	37.6	11.7	25.9
48.3	41.2	12.4	28.8
72.2	44.7	21.0	23.7
96.2	46.7	24.6	22.1
120.0	53.8	32.2	21.6
144.5	56.7	43.1	13.6
192.4	65.8	50.7	15.1
264.0	68.6	64.8	3.8
336.0	69.5	69.8	-0.3

*Swede.*

The discs were cut on March 14 and washed in aerated running tap-water for 24 hours before transference to 0.001 M manganese chloride. Samples of

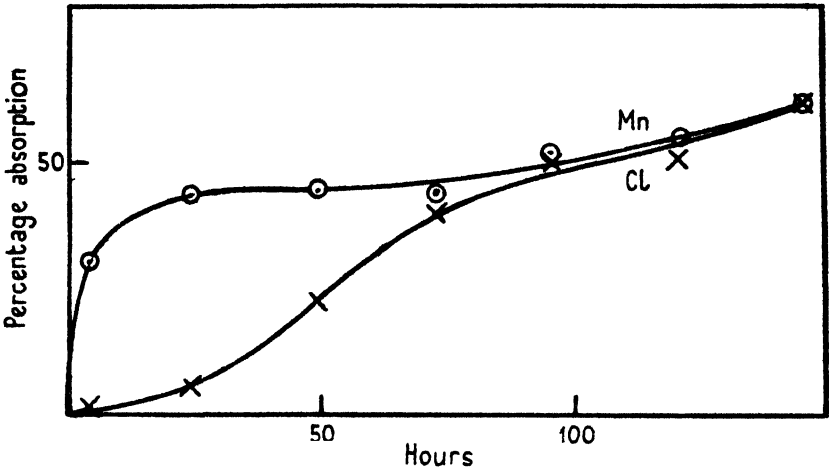


FIG. 2. The course of absorption by swede-root tissue of the ions of manganese chloride from a solution of an initial concentration of 0.001 M.

TABLE V  
*Course of Absorption of the Ions of Manganese Chloride by Swede*

Hours.	Absorption of Mn (% of original quantity in external solution).	Absorption of Cl (% of original quantity in external solution).	Mn—Cl.
4.0	29.5	1.3	28.2
24.0	42.4	5.0	37.4
48.0	43.7	22.3	21.4
72.0	42.7	39.7	3.0
95.25	50.4	49.8	0.6
120.0	53.3	50.5	2.8
144.0	60.9	61.3	-0.4

the external solution were withdrawn at intervals and both manganese and chloride determined polarographically. The course of absorption is shown by the data given in Table V and by the curves in Fig. 2. The temporary decline in the rate of absorption of manganese at the end of the initial period of rapid absorption is clearly shown. Equality of the total amount of absorption of manganese and chloride was reached in about 100 hours.

### Mangold.

Discs were cut from roots of mangold (Red Intermediate) on May 25 and washed in aerated running tap-water for 24 hours before transference to

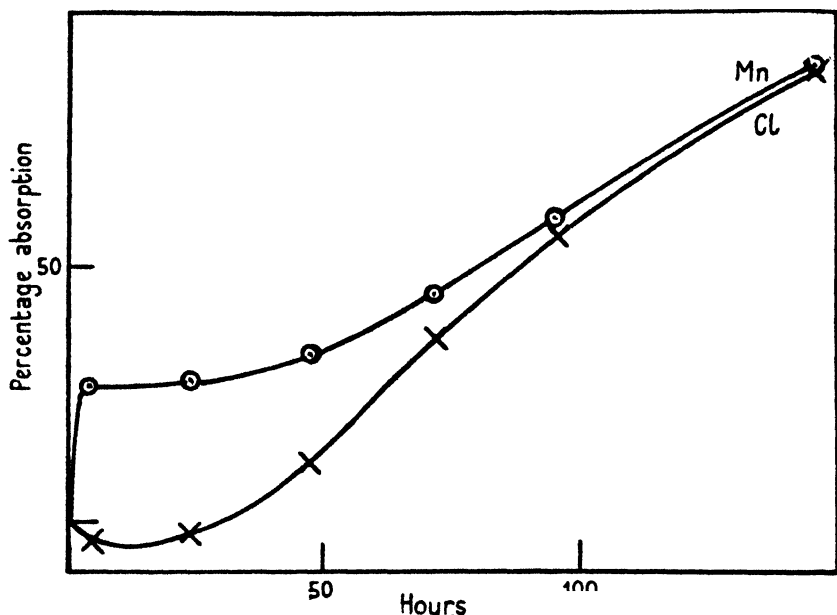


FIG. 3. The course of absorption by mangold-root tissue of the ions of manganese chloride from a solution of an initial concentration of 0.001 M.

0.001 M manganese chloride. Samples of the external solution of 10 replicate experiments were withdrawn after various times; the manganese was determined by the periodate method and the chloride polarographically. The course of absorption of the two ions is shown by the data in Table VI (experiment 46) and graphically in Fig. 3. Both temporary slowing down in the rate of manganese absorption and the final equality of total absorption of manganese and chloride are clearly exhibited.

It will be observed that there is at first an exosmosis of chloride from the tissue which gives place after more than 24 hours to absorption. This phenomenon of exosmosis from the tissue of ions present in the external solution has already been noted in the work on potassium salts described in the first paper of this series. The possible significance of this will be discussed later.

A second experiment with discs of mangold (Red Intermediate) cut on June 26 and washed in aerated running tap-water gave similar results. The experimental data (experiment 47) are recorded in Table VI.

TABLE VI  
*Course of Absorption of the Ions of Manganese Chloride by Mangold*

Hours.	Absorption of Mn (% of original quantity in external solution).	Absorption of Cl (% of original quantity in external solution).	Mn—Cl.
<i>Expt. 46</i>			
4	27.2	-3.4	30.6
24	27.6	-2.4	30.0
48	32.3	12.0	20.3
72	44.3	36.3	8.0
96	59.6	56.5	3.1
146	89.8	88.2	1.6
<i>Expt. 47</i>			
4	24.25	-0.7	24.95
24	31.5	-4.6	36.1
48	34.8	6.7	28.1
72	40.6	33.8	2.8
96	61.4	61.4	0.0

*Red beetroot.*

Discs of freshly harvested red beetroot (Sutton's Crimson Globe) were cut on July 24 and washed for 23.5 hours in aerated running tap-water and then for 0.5 hour in four changes of distilled water. The discs were then transferred to 0.001 M manganese chloride, 8 replicates being used. Samples were extracted from the external solution at intervals; the samples taken from all the replicates at one time were put together and used for a single determination of manganese by the periodate method and for determination of chloride polarographically. The results are shown in Table VII (experiment 49) along with those of a second experiment (experiment 50) with discs cut

TABLE VII  
*Course of Absorption of the Ions of Manganese Chloride by Red Beetroot*

Hours.	Absorption of Mn (% of original quantity in external solution).	Absorption of Cl (% of original quantity in external solution).	Mn—Cl.
<i>Expt. 49</i>			
4	19.9	3.6	16.3
24	31.2	21.6	9.6
48	43.2	36.7	6.5
72	59.6	46.5	13.1
96	62.2	51.8	10.4
144	73.8	71.7	2.1
<i>Expt. 50</i>			
4	24.45	6.65	17.8
26	33.7	23.35	10.35
48	45.4	40.6	4.8
72	57.4	51.3	6.1
96	66.9	67.95	-1.05
156	73.8	74.25	-0.45

from a freshly harvested root on July 31 and treated in the same way as those used in the earlier experiment. The results for experiment 50 are shown graphically in Fig. 4. It is clear that the absorption of manganese chloride by tissue of red beetroot follows a course closely similar to that by mangold.

It can be concluded, therefore, that the course of absorption of manganese chloride from dilute solutions by all the tissues, carrot, swede, mangold, and red beetroot, is similar and is, in fact, that described in the previous paper of this series for the absorption of manganese salts by carrot-root tissue.

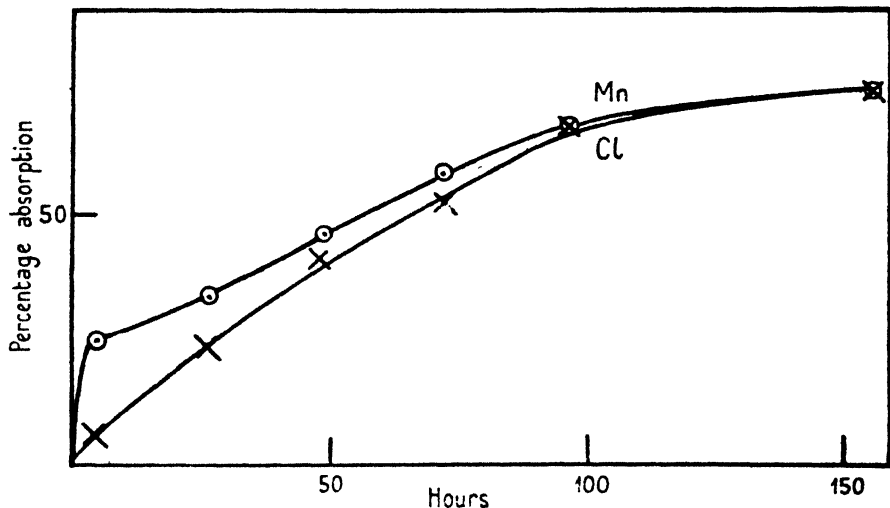


FIG. 4. The course of absorption by red beetroot tissue of the ions of manganese chloride from a solution of an initial concentration of 0.001 M.

#### THE EFFECT OF PROLONGED WASHING IN AERATED RUNNING TAP-WATER ON THE ABSORPTION OF MANGANESE CHLORIDE BY STORAGE TISSUE

A few references are to be found in the literature of salt absorption indicating that prolonged washing in tap-water brings about an enhanced absorption of ions. Thus Asprey (1937) found that the amounts of both ions of ammonium chloride absorbed by potato tissue from dilute solutions in 24 hours at 25° C. were increased with increase in the time of washing in running tap-water for periods up to 191 hours. Steward and Harrison (1939) state that Steward and Berry found that after washing in running tap-water for 1, 25, and 50 hours potato tissue placed in 0.00075 M potassium bromide at 23° acquired in 48 hours sap concentrations of 5.81, 13.04, and 18.94 mg. equivalents per litre respectively. The experiments described below show very definitely the favourable effect on ion uptake of prolongation of the washing period.

##### *Carrot.*

Discs of carrot were cut on April 17 and transferred to aerated running tap-water. Samples of tissue were removed after 23, 47.8, and 95.2 hours and



transferred to 0.001 M manganese chloride at 25° C. The concentration of manganese in samples withdrawn after various times was determined polarographically. The results are shown in Table VIII.

They show that the absorption of manganese by carrot tissue is definitely increased with increase in the time of preliminary washing in tap-water.

TABLE VIII  
*Effect of Length of Preliminary Washing on the Absorption of Manganese from 0.001 M Manganese Chloride by Carrot Tissue*

Hours of washing.	Hours from immersion in 0.001 M $\text{MnCl}_2$ .	Percentage of Mn in original solution absorbed.
23	9.65	27.2
	24.0	30.2
	48.6	39.3
	72.4	40.9
	96.0	43.5
	144.2	50.5
	264.1	53.4
47.8	3.65	30.2
	24.2	32.4
	48.0	47.7
	72.0	53.2
	120.05	55.6
95.2	4.0	32.2
	24.0	46.7
	46.6	50.6
	72.0	57.3
	120.8	63.6

#### *Red beetroot.*

More definite evidence was obtained with this tissue. Discs were cut on December 3 and transferred to aerated running tap-water. Samples of tissue were removed from the tap-water to 0.001 M manganese chloride at 25° C. after 22, 94, and 166 hours. Samples of the external solutions were withdrawn at various times and the concentrations of manganese and chloride in the samples determined polarographically. The results are given in Table IX and graphically in Fig. 5.

It will be observed that the characteristics of the absorption of manganese chloride already noted again appear. Again there occur the two phases in the absorption of the cation with the temporary fall in the rate of absorption as the first phase passes into the second. Again the gradual approach to equality of absorption of manganese and chloride during the second phase is clear. But other facts are also disclosed. The most obvious of these is that the longer the preliminary washing in tap-water the sooner the second phase of absorption sets in. One result of this is that the period of temporary lowering of absorption rate at the end of the first phase of absorption is diminished with increased length of the washing period. Thus, in the experiments with red beetroot with 22 hours' preliminary washing the subsequent rise in the rate of manganese absorption did not appear until more than 40 hours after immersion

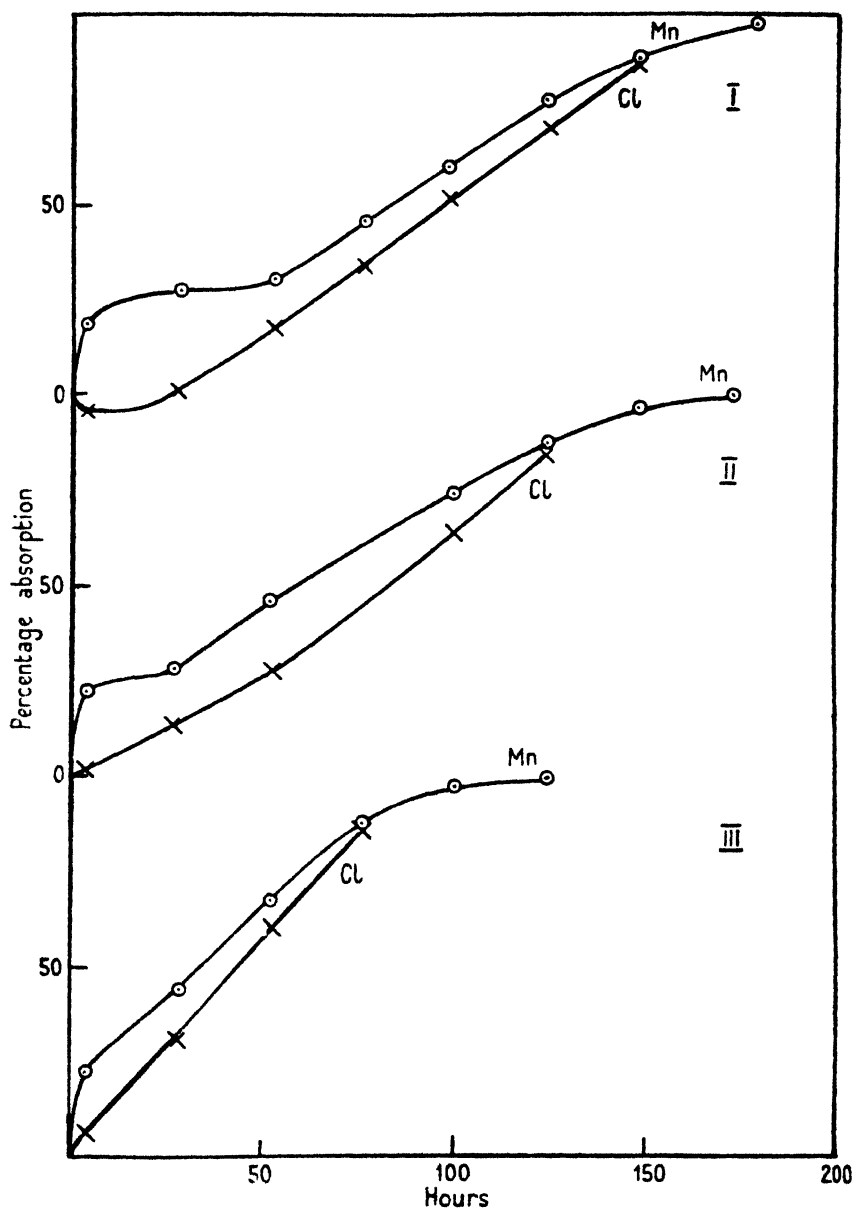


FIG. 5. The course of absorption by red beetroot tissue of the ions of manganese chloride from a solution of an initial concentration of 0.001 M. I, after preliminary washing in aerated running tap-water for 22 hours; II, after preliminary washing for 94 hours; III, after preliminary washing for 166 hours.

TABLE IX

*Effect of Length of Preliminary Washing on the Absorption of Manganese and Chloride from 0.001 M Manganese Chloride by Red Beetroot Tissue*

Hours of washing.	Hours from immersion in 0.001 M $\text{MnCl}_2$ .	Absorption of Mn (% of original quantity in external solution).	Absorption of Cl (% of original quantity in external solution).	Mn—Cl.
22	4.0	17.2	-4.3	21.5
	28.0	27.1	0.7	26.4
	52	29.9	18.3	11.6
	76	45.4	34.0	11.4
	98	59.5	51.4	8.1
	124	76.15	69.9	6.25
	148	89.1	86.6	2.5
	178	97.9		
94	4.2	22.0	2.2	19.8
	26.5	28.4	13.7	14.7
	52	45.15	27.5	17.65
	100	73.2	63.6	9.6
	124	87.6	84.2	3.4
	148	96.85		
166	172	99.7		
	4.0	21.2	6.1	15.1
	28.0	43.4	30.4	13.0
	52	67.4	60.0	7.4
	76	88.1	85.6	2.5
	100	96.7		
	124	99.6		

of the tissue in the manganese solution; with 94 hours' preliminary washing this time was reduced to about 20 hours and with 166 hours' washing it had disappeared. Similarly with the anion, with the shortest period of washing there was first a slight exosmosis of chloride before absorption took place and a steady intake of chloride at an approximately constant rate appeared to begin after about 30 hours, but with longer periods of washing absorption appeared to begin immediately or very soon after immersion of the tissue in the salt solution. Not only is the onset of the second phase of absorption speeded up by washing in running tap-water, but with prolonged washing the rate of absorption of both ions during this phase appeared to be increased. Under the conditions of these experiments the rate of absorption during the greater part of the second phase is approximately constant, and this applies to both ions.

#### DISCUSSION OF EXPERIMENTAL RESULTS

Certain facts regarding the absorption of manganese chloride by thin slices of storage tissue are now clear. After a preliminary period of washing in aerated running tap-water for about 24 hours, such tissue, when placed in a dilute (0.001 M) solution of manganese chloride, immediately absorbs manganese at a rapid rate, whereas the absorption of chloride proceeds very slowly for 24 to 48 hours. Sometimes there may even be exosmosis of chloride into the external solution, although whether this occurs at all, or the extent of it

if it does occur, probably depends on the chloride content of the tissue. This first phase of absorption tends towards a position of equilibrium as far as the manganese is concerned, but such a position is generally never reached, at any rate when the tissue is adequately supplied with oxygen, because a second phase in absorption supervenes in which absorption of both ions continues at a steady rate which noticeably declines only with the approaching exhaustion of the salt in the external liquid. Since, with declining external concentration, the rate of absorption remains approximately constant, it must be concluded either that within limits the absorption process is independent of the concentration (cf. Robertson, 1941) or that some internal factor furthering absorption is increasing and that this increase approximately balances the tendency for the absorption rate to fall on account of the lowering of the external concentration. That the latter may be the explanation appears from the fact that the rate of absorption does actually fall before complete exhaustion of the salt in the external solution.

Prolonging the period of preliminary washing has a very definite effect on the absorption of both ions. The data so far obtained suggest that the first phase of rapid cation absorption is not affected to any very significant extent, but further work is necessary to decide this point. It is the second phase of long-continued absorption of both ions that is markedly influenced by the length of the washing period. The longer the washing with aerated running tap-water (at a temperature of 10° to 14° C.) the sooner the second phase becomes significant, and, at any rate with prolonged washing, the more rapid the absorption. Indeed, after washing for 166 hours the lag period in the onset of the second phase of absorption disappeared.

It is reasonable, as indicated in the previous paper of this series, to ascribe the two phases of absorption to two different processes. The first phase, in which absorption of the cation takes place rapidly towards a position of equilibrium with no corresponding rapid absorption of anion, might be conditioned by adsorption, Donnan equilibrium, or both. Arguments have been advanced in the previous paper in this series in support of the view that Donnan equilibrium is likely to be more important than adsorption in bringing about the initial rapid intake of manganese. The operation of Donnan equilibrium will also explain the exosmosis of chloride from tissue into dilute solutions of manganese chloride. Thus let us suppose the original quantity of manganese and chloride ions in the external solution is  $a$  (counting each divalent manganese ion as 2) and that in the tissue there is a quantity of free potassium ions  $b$ , balanced partly by a quantity of chloride ions  $c$ , and protein or other immobile anions  $b-c$ . Let us further suppose the volume of the external solution is 20 times the effective volume of the tissue fluid, so that the external ions are distributed through 20 times the volume occupied by the solution in the tissue. Then the conditions for Donnan equilibrium are given by the equations

$$0.0025(a-x)(a+y) = x(c-y), \quad (1)$$

$$0.0025(x+y)(a+y) = (b-x-y)(c-y), \quad (2)$$

where  $x$  is the quantity of manganese which diffuses into the tissue,  $y$  the quantity of chloride which diffuses out of the tissue, and  $x+y$  the quantity of potassium which diffuses out of the tissue. Solving equations (1) and (2) for  $y$  we have

$$y = \frac{(0.005a+b+c) \pm \sqrt{\{(0.005a+b+c)^2 - 3.99(bc - 0.0025a^2)\}}}{1.995} \quad (3)$$

The solution with the term  $\sqrt{\{(0.005a+b+c)^2 - 3.99(bc - 0.0025a^2)\}}$  positive is inadmissible as it can give values for  $y$  greater than  $c$ , hence the quantity of chloride diffusing out of the tissue is given by

$$\frac{(0.005a+b+c) - \sqrt{\{(0.005a+b+c)^2 - 3.99(bc - 0.0025a^2)\}}}{1.995}$$

This will be positive, that is, chloride will diffuse *out* of the tissue, so long as  $bc - 0.0025a^2$  is positive, or  $bc > 0.0025a^2$ . That is, if the product of the concentrations of chloride and potassium ions in the tissue is greater than the product of the concentrations of manganese and chloride ions outside the tissue (each manganese ion counting as 2), chloride will diffuse out of the tissue into the external solution. If, on the other hand,  $bc < 0.0025a^2$ , chloride will diffuse into the tissue from the external solution.

To take an example showing the extent of ionic exchange resulting from these conditions let us suppose that the quantity of monovalent potassium ions in the tissue is double the number of divalent manganese ions in the external solution and that 90 per cent. of the potassium ions in the tissue are balanced by immobile ions and the rest by chloride ions, so that  $a = b = 10c$ . It will be observed that  $bc > 0.0025a^2$  and so chloride ions will diffuse out of the tissue. Solving equations (1) and (2) above we have  $y = 0.0967a$  and  $x = 0.453a$ ; that is about 45 per cent. of the manganese is absorbed by the tissue in the establishment of equilibrium, while the concentration of chloride in the external solution rises by about 9.7 per cent.

In actual fact it is likely that more than one cation will be present in the tissue and other mobile anions besides chloride, which indeed is not necessarily present at all. The argument is not affected if the cations are of more than one kind, but if there are other mobile anions as well as chloride in the tissue the matter is more complicated. If the original concentration of cations is again  $b$  while the concentration of chloride and other anions are  $c$  and  $d$  respectively (all reckoned as univalent), the quantity of chloride which has diffused out of the tissue when equilibrium is attained is given by the expression

$$\frac{(a+c)}{(a+c+d)} \left[ \frac{(0.005a+b+c+d) \pm \sqrt{\{(0.005a+b+c+d)^2 - 3.99[b(c+d) - 0.0025a^2]\}}}{1.995} - \frac{ad}{a+c} \right] \quad (4)$$

which, of course, reduces to the expression (3) above when  $d = 0$ .

It is of interest to consider a few theoretical cases. First let us suppose that the quantities of ions are the same as in the instance considered above where

the only mobile anion was chloride, except that there is in the tissue a quantity of mobile anion other than chloride, but equal to it in amount, so that  $a = b = 10c = 10d$ . Substitution in the expression (4) gives the value of chloride which diffuses out of the tissue as 95.6 per cent. of the total amount; so that the chloride concentration of the external solution rises by 9.56 per cent. If the amount in the tissue is reduced to half and other anions increased to 5 times that in the instance just given, then the amount of chloride which diffuses out is 82.7 per cent. of the chloride originally in the tissue and the concentration of chloride in the external solution rises by only about 4.1 per cent.

The above examples are sufficient to show that, provided chloride ions are present in the tissue, an exosmosis of such ions into the external solution of manganese chloride can occur. Similarly it can be shown that the exosmosis of both potassium and chloride ions from tissue into dilute solutions of potassium chloride, such as was shown by Stiles and Skelding (1940), can be expected. The absence of such exosmosis in higher concentrations of potassium chloride is also explained.

The establishment of a Donnan equilibrium on the lines indicated above involves an exchange of ions between the external solution and the tissue. That such an exchange does indeed take place was shown by one of us more than twenty years ago (Stiles, 1924).

It should be mentioned that whereas the observed facts regarding the first stage in the absorption of manganese chloride by storage tissue can be adequately explained in terms of the establishment of a Donnan equilibrium, adsorption may play some part in the absorption of manganese. Arguments were, however, given in the previous paper in this series to show that adsorption alone cannot explain the absorption of manganese salts by storage tissue.

On the view here put forward then, the transference of discs of storage tissue to a solution of manganese chloride is followed by an exchange of ions between tissue and external solution mainly conditioned by Donnan equilibrium, and as a result of which the external solution now contains ions from the tissue in addition to the original manganese and chloride ions. An actual position of equilibrium is, however, rarely if ever attained because of the setting in of the second phase of absorption during which both cations and anions are absorbed until, with dilute solutions, exhaustion of salt in the external medium is approached. As the chloride is absorbed at a somewhat more rapid rate than the manganese, it is clear that the cations which diffused out from the tissues in exchange for manganese ions are reabsorbed, since cation and anion must enter the tissue, and remain in the external solution, in equivalent quantities.

These findings fall into line with those of Stiles (1927) on the behaviour of storage tissue in distilled water. It may be recalled that, provided the tissue is adequately supplied with oxygen, when thin discs of storage tissue are placed in distilled water there is at first an exosmosis of ions from the tissue which is followed by a long-continued absorption of the ions back into the tissue. It was originally thought that the initial increase of ionic concentration

of the external medium was due to the the diffusion of ions from the dead or dying cut or superficial cells, but in view of later work it would seem more reasonable to suppose that here also we are concerned with the establishment of a Donnan equilibrium, followed by long-continued absorption.

The characteristic of what is here called the second phase of absorption, the continued absorption of both cations and anions from the external solution with consequent loss of salt from the latter and an apparent accumulation of salt in the tissue against the concentration gradient, was shown by Stiles and Kidd in 1919 for thin potato discs immersed in a number of salts by measurements of the electrical conductivity of the solutions and has since been abundantly confirmed by both conductivity and other measurements (see e.g. Stiles, 1924; Steward, 1932; Steward and Harrison, 1939; Stiles and Skelding, 1940, 1940a; Robertson, 1941).

Nevertheless, no generally acceptable explanation of the mechanism of this absorption has so far been put forward. The simplest explanation of such absorption would be chemical combination of the absorbed salt with, or its adsorption by, some cell constituent, the rate of the process being partly determined by diffusion. What would be the actual course of absorption in such conditions is difficult to say, since the conditions of absorption by a cylinder through its whole surface are mathematically very complex. It would appear likely that if absorption is indeed due to chemical action, the rate of the reaction is controlled by some internal factor at present unknown. If the absorption is due to adsorption, this, as one of us has already pointed out (Stiles, 1938), can only be at the first stage in the process, and Robertson (1941, 1944) has now developed a theory of salt absorption, based on the results of experiments with discs of carrot tissue, in which the first stage of salt accumulation is held to be adsorption in the outer region of the cell to a constant concentration independent of external concentration, this stage being followed by the progressive accumulation of the salt in an inner region of the cell. The existence of these two stages is indicated by the observation of S. C. Brooks (1937), who by working with a radio-active isotope of potassium showed that potassium ions accumulated in the cytoplasm before accumulating in the vacuole.

It is generally supposed that absorbed ions accumulate as such in the vacuole, although arguments advanced in favour of this view are not always very convincing. Among such are data on the ionic concentration of expressed sap, for since the tissue is necessarily killed in obtaining the sap there is no guarantee that ions which are free after the cells are killed are in this condition in the living cell. It is not unlikely that killing results in the breaking down of complexes with the release of simple substances which exhibit ionic dissociation. However, if we provisionally accept the view that accumulation of ions does indeed take place in the vacuole, we are faced with the fact that energy is necessary to move the accumulated ions against a concentration gradient. The obvious source of this energy is, of course, to be found in respiration, and ever since Lundegårdh and Burström (1933) put forward their theory of anion respiration

and Steward (1932) emphasized the importance of oxygen supply for the accumulation of salt, much attention has been given to the possible relationship between absorption of salt and respiration by these workers and others, particularly Robertson (1941, 1944).

We do not intend to enter into a general discussion of this question at this stage. We would, however, refer to our findings in respect to the effect of prolonged washing in aerated running tap-water on salt uptake. It will be recalled that extending the washing period from 1 to 4 days has the effect of speeding up the onset of the phase of salt accumulation and with further extension of washing to 7 days, not only is the lag period removed but the rate of absorption is also increased. Now extended washing in aerated running tap-water has a very definite effect on the respiratory activity of thin slices of storage tissue. Bennet-Clark and Bexon (1943) found a continuous rise in the respiration rate of beetroot slices during such washing for about 300 hours, the respiration rate rising from about 30  $\mu$ l. of oxygen per hour per gm. of fresh weight shortly after cutting to about 130  $\mu$ l. of oxygen per hour per gm. of fresh weight at the end of 300 hours' washing. We have confirmed this effect of continued washing in aerated running tap-water for a number of storage tissues (Stiles and Dent, 1946) and suggested that it may result from the development of some part of the respiratory system in tissues previously under very low oxygen and high carbon-dioxide tension when, on cutting out of a storage organ, these are exposed to oxygen, for the development of a high respiration rate occurs much more rapidly when the discs are exposed to air at 25° C. instead of aerated running tap-water at 10° to 14° C. However this may be, it would appear that the development of the capacity for absorbing salt goes along with the development of respiratory activity. While this finding does not help us to decide whether the connexion between salt accumulation and respiration is direct or remote, it does indicate that the enhanced salt absorption with prolonged washing is related to the increased metabolic activity of the tissue in general, and so to what Lundegårdh and Robertson have called 'ground respiration' and not to the increment in the respiration which results from the presence of the salt and which Lundegårdh called 'anion respiration' and Robertson 'salt respiration', and which, in experiments with discs of storage tissue such as those described in this and Robertson's work, is supposed to provide the energy for salt accumulation.

We would conclude this discussion with a few words about the lag or induction period which is generally observed between the immersion of discs of tissue in a salt solution and the onset of the phase of accumulation. That this is not merely a masking of the accumulation process by the phase of ionic exchange is shown by (1) the observation of Steward and Harrison (1939) that the absorption of bromide from rubidium bromide by potato exhibited the phenomenon and there could be no exosmosis of bromide from the tissue since the latter does not contain this ion, and (2) the slowing down in the absorption of the cation so frequently shown between the first phase of absorption, that of ionic exchange and the second phase, that of accumulation.



Robertson (1944) did not find any preliminary lag period before the uptake of potassium chloride by carrot discs and concludes from this, and from his finding a non-linear relation between time and intake of salt, that accumulation at different temperatures in potato is 'clearly different from accumulation in carrot tissue'. From our own observations with carrot tissue we cannot agree with this, for under certain conditions carrot, as well as beetroot and mangold, shows a lag period in accumulation as marked as that found for potato. Whether the lag period occurs or not would appear to depend on the general level of metabolic activity of the tissue, which is increased by prolonged washing in aerated tap-water. Steward and Harrison (1939) found the lag period in the absorption of rubidium bromide by potato discs was reduced from about 20 hours to 3.67 hours by pre-treating the discs for 24 hours with aerated water at 23°C. instead of running tap-water below 10°C., and they conclude that the lag is due to the time taken for the tissue to acquire the necessary high metabolic rate, a conclusion which is in agreement with our own. By pre-treating his discs with distilled water until the electrolytes which diffused out were reabsorbed, Robinson removed the lag period. The lag or induction period would thus seem to correspond to a time during which the metabolic activity of the tissue is increasing, and on this point it may be recalled that Steward and Preston (1941) found that potassium chloride increased the respiration rate of potato discs, although they found calcium chloride lowered it, Robertson (1941) found the respiration rate of carrot discs increased by chlorides of potassium, sodium, lithium, calcium, and magnesium, and Bennet-Clark and Bexon (1943) found both potassium chloride and calcium chloride increased the respiration rate of red beetroot discs. The increase in respiratory activity by dilute solutions of inorganic chlorides would thus seem to be very general.

It can then be concluded that when thin slices of storage tissue, previously washed in aerated running tap-water at about 12°C. for one day, are transferred to a dilute solution of manganese chloride, the sequence of events is as follows. At first there is a rapid absorption of manganese which may be accompanied, if the tissue contains an appreciable amount of chloride, with exosmosis of chloride ions. This phase involves an exchange of cations in the tissue for manganese ions and can be interpreted as the establishment of a Donnan equilibrium. The condition of equilibrium, at any rate with dilute solutions, is scarcely ever attained, as the second phase of active absorption sets in after a longer or shorter lag or induction period in which all ions now present in the external solution are absorbed. The onset of this phase corresponds with the development of increased metabolic activity as indicated by an increase in respiration rate, the respiration rate of discs of storage tissue being low when cut out of the storage organs but increasing with prolonged washing in aerated tap-water or when exposed to dilute salt solutions. The lag period is thus reduced by increasing the length of the washing period. It can probably also be reduced by raising the temperature of the washing water.

#### SUMMARY

The absorption of manganese by thin discs of carrot and mangold roots from 0.001 M solutions of manganese chloride after various times was examined by (1) polarographic determination of manganese in the external solution and (2) by estimation by means of the periodate method. There was no significant difference between the results given by the two methods, but the polarographic method is the more sensitive and probably the more accurate of the two methods.

The absorption of chloride from the same solutions by the same tissue was determined (1) by polarographic estimation of chloride in the external solution, (2) by titration of the external solution with silver nitrate with the use of dichlorofluorescein as adsorption indicator, (3) by ashing the tissue at a low temperature, extracting with water, and titrating the extract with silver nitrate with the use of the adsorption indicator. The results obtained in all three ways showed adequate agreement with one another. Since very much less material is required for the polarographic estimation of chloride this method is preferable to the titration method where it is advantageous or necessary to use as small an amount of material as possible.

Where, in work on salt absorption by plant tissue, the investigator has the choice of analysing either the external solution or the tissue, the former is preferable. The solutions contain a very small amount of impurity, whereas the element to be determined is likely to comprise only a small fraction of the total plant material. Further, analysis of the solutions, since it does not involve the destruction of the tissue, allows the use of very much less material.

The course of absorption of the two ions of manganese chloride from 0.001 M solutions of this salt by thin slices of roots of carrot, swede, mangold, and red beet has been examined. The results obtained confirm those previously recorded for the absorption of this salt by carrot. With all the tissues examined, after a preliminary washing for about 24 hours in aerated running tap-water the absorption of manganese shows a two-phase course, an initial rapid absorption of manganese which tends towards an equilibrium after a few hours being followed by a long-continued absorption of the ion. During the first phase the absorption of chloride is slight and there may even be a diffusion of chloride out of the tissue, but this gives place during the second phase to long-continued absorption.

Arguments are put forward to show that the first phase of absorption involves an exchange of ions mainly conditioned by Donnan equilibrium. In the second phase all the ions by then present in the external solution are absorbed so that, as originally shown by Stiles and Kidd 27 years ago for a number of chlorides, there is a continued loss of salt from the solution, and accumulation of salt takes place in the tissue.

The lag or induction period between the immersion of tissue in the solution and the beginning of absorption of both ions at an appreciable rate is lessened or eliminated by prolonged washing in aerated running tap-water. Such treatment brings about an increase in metabolic activity as indicated by increase in respiration rate, and it is therefore likely that the lag period occurs

while the metabolic activity is rising to a level necessary for accumulation to take place. This rise is probably also induced by rise in temperature of the washing water.

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# Experimental and Analytical Studies of Pteridophytes

## IX. The Effect of Removing Leaf Primordia on the Development of *Angiopteris evecta* Hoffm.

BY

C. W. WARDLAW

(Department of Cryptogamic Botany, University of Manchester)

With Plate VI and fourteen Figures in the Text

### INTRODUCTION

THE periodic interruption of the conducting cylinder in leptosporangiate ferns is associated with the 'insertion' of the leaf-traces, i.e. the vascular strand or strands of the petiole or leaf-base. Further light may be thrown on this relationship by experimental means. Thus, in *Dryopteris aristata* and *D. filix-mas*, if successive leaf primordia are destroyed at a very early stage, the vascular system of the shoot does not develop as a dictyostele but as a continuous uninterrupted cylinder or solenostele (Wardlaw, 1944a). Other observations indicate how this result can be related to the facts of stelar development in the growing region (Wardlaw, 1945). If young leaf primordia of *Osmunda regalis* are destroyed an uninterrupted cylinder of xylem develops in the experimental region of the shoot, in contrast to the normal dictyoxyletic development in the untreated region lower down (Wardlaw, 1946). These data also show that although the decurrent leaf-traces contribute to the vascular system of the shoot during the normal development, the shoot stele in *Dryopteris* and *Osmunda* is essentially axial in origin.

It is desirable that these findings should be tested on as broad a basis as possible. Accordingly a study has been made of the effect of removing successive leaf primordia from the shoot apex of *Angiopteris evecta* Hoffm. It was considered that this experiment might prove instructive from several points of view. As *Angiopteris evecta* is a primitive eusporangiate fern, it is a matter of interest to know (i) how it compares with a leptosporangiate fern such as *Dryopteris aristata* when subjected to comparable experimental treatment, and (ii) the effect of continuous defoliation on its large, polycyclic, dictyostelic vascular system. The classical anatomical investigations of the Marattiaceae indicate that the decurrent leaf-traces constitute the greater part of the vascular tissue of the shoot, the cauline or axial contribution being of meagre extent. In very young sporophytic plants Campbell (1921) has suggested that no cauline vascular tissue is present, the stele being a composite structure composed entirely of fused decurrent leaf-traces. It will be a matter of interest to ascertain whether the experimental data confirm or refute these

conclusions. Physiological aspects of defoliation, the phenomenon of attenuation, the relation of the apical meristem to the initial differentiation of vascular tissue, and other observations are also described and discussed.

#### MATERIALS AND METHODS

The material used has included young sporophytic plants of different ages, a large lateral bud from an old plant, and plants of different ages produced by placing isolated petiole bases in moist peat in a germinator. As Van Leeuwen (1912) has shown, the fleshy, stipulate leaf-bases may each produce four new buds or plantlings. The experimental treatment was as already described (Wardlaw, 1944a), the distal ends of shoots being completely defoliated and all new leaf primordia dissected off over a period of weeks or months. But whereas in *Dryopteris aristata* this is a relatively simple operation, in *Angiopteris evecta* it is otherwise; the removal of the fleshy stipulate leaf-bases, with their extensive development in the transverse plane, calls for very careful dissection in order to avoid injuring the flat and somewhat inconspicuous apical meristem. The experimental plants were placed in moist peat and kept in a germinator at 22°–25° C.

#### OBSERVATIONS ON DEFOLIATED PLANTS

A lateral bud of about 2 cm. diameter was removed from the parent plant and defoliated as described above (May, 1944), all new leaf primordia being removed at intervals of 10–14 days. The plant became rooted in the peat. After some time the superficial area of the apical meristem became noticeably reduced. At the end of 4 months of continuous defoliation the distal region of the shoot had grown out as a small leafless cone, and the apical meristem—characterized at an earlier stage as a turgid, shiny cushion of tissue—could no longer be distinguished. The terminal region of the shoot, in fact, had the appearance of superficial parenchymatous tissue. The specimen was kept under observation for a further period of one month. During that time the apical region remained green and healthy, but there was no further development of primordia and no evidence of growth (Pl. VI, Fig. 1). At this stage the specimen was fixed, embedded in wax, and sectioned transversely.

In the thick basal region of this specimen a polycyclic stele was present, the inner ring consisting of several meristeles (Text-fig. 6). As a result of the continuous defoliation the diameter of the shoot diminished rapidly with a concomitant reduction in the size and complexity of the vascular system, till eventually, in the distal region, the stele consisted of a small solid core of tracheides (Text-figs. 1–6 and Pl. VI, Figs. 2–4). As the illustrations show, a feature of this reduction was the fading out of individual meristeles: in the first stage the xylem failed to become differentiated and lignified, and this was followed by the gradual decrease and eventual disappearance of the small-celled phloem and vascular parenchyma. A parallel was thus afforded with the disappearing meristeles already described in attenuated plants of *Onoclea sensibilis* (Wardlaw, 1945). Near the distal region of the shoot the two remaining



TEXT-FIGS. 1-6. Transverse sections of an experimental plant, Pl. VI, Fig. 1, showing the progressive reduction in the size and complexity of the vascular system consequent on the removal of all young leaf primordia. Text-fig. 1. Protoste in the distal apical region. Text-figs. 4, 5. Stages in stelar reduction. Xylem, solid black; periphery of stele, continuous line. ( $\times 22$ .) Text-fig. 6. Polycyclic, dictyostelic condition in the older region of the shoot. ( $\times 22$ .)

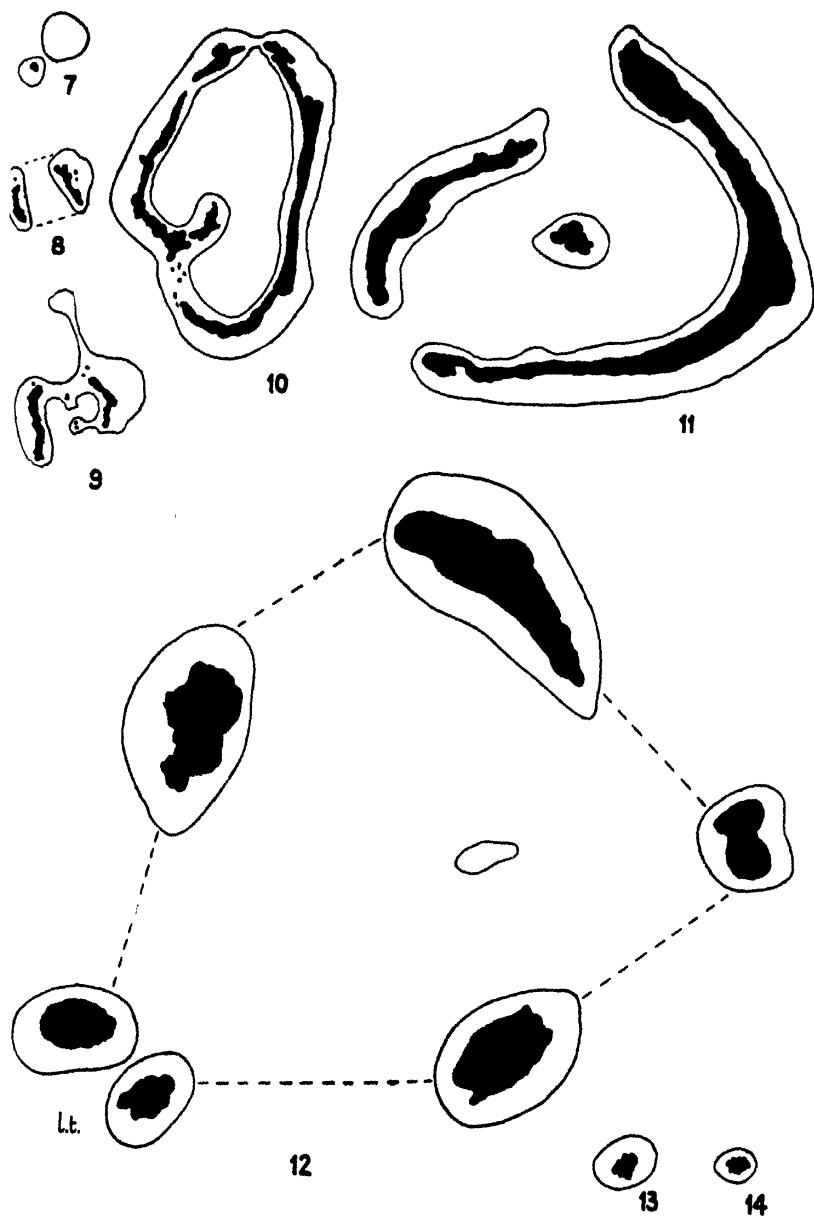
meristemes (Text-fig. 3) became conjoined so that a single protostele of approximately radial symmetry occupied the centre of the shoot. This protostele, Pl. VI, Fig. 3, also faded out about 1 mm. below the apex (Pl. VI, Fig. 2), the most distal region being occupied by parenchymatous tissue comparable in cell-size and appearance with the normal cortical parenchyma. No trace remained of the distinctive cells of the apical meristem. About the level where the distal protostele began to fade out small quantities of starch were present in the tissue surrounding the incipient vascular tissue. (The endodermis could not be distinguished in these preparations.) Starch was also present in increasing quantities in the inner cortex and pith on proceeding downwards in the shoot, though the total amount present was never great. Starch was consistently absent from the outer cortex.

Because of the rapid diminution in the size of the stele and the eventual cessation of growth at the apex, the primary object of this experiment was not realized.

In a second experiment defoliation was systematically carried out on a well-rooted young sporophyte plant in its second year of growth. As before, the apical meristem progressively diminished in surface area and eventually new primordia ceased to be formed or were formed very slowly. At this stage the distal region of the shoot, as in the previous specimen, had grown out into a leafless cone (Pl. VI, Fig. 6), the residual apical meristem being occluded in a small depression. The specimen was kept under observation for a further period of 6 weeks, during which time no further apical growth took place. At this stage the material was fixed, embedded, and sectioned transversely.

Text-fig. 12 shows the appearance of the vascular system in the thickest part of the shoot. Above this level, in the defoliated region, the vascular system underwent a considerable reduction in diameter. In this plant the effect on the shoot-stele of destroying the young leaf primordia was clearly apparent in that an approximately uninterrupted vascular ring, i.e. a solenostelic condition, had been induced (Text-figs. 10, 11; Pl. VI, Figs. 7, 8). Where the leaf primordia had not been destroyed at a sufficiently early stage leaf-gaps of varying size were present. In the distal region of the shoot the stele underwent a rapid decrease in size, individual meristemes fading out till finally only two remained (Text-figs. 8, 9). In proximity to the apical meristem one of these strands was seen to be confluent with, or the downward extension of, a leaf-trace; the other was the axial stele which originated below the apical meristem (Text-fig. 7). In the distal, somewhat lateral, depression the distinctive cells of the apical meristem could be observed. A small leaf primordium, to which the trace mentioned above belonged, was associated with the apical meristem.

These observations afford further evidence of the relation between leaf development and the morphology of the shoot-stele already demonstrated in *Dryopteris* (Wardlaw, 1944a). They also show that rapid attenuation of the shoot attends defoliation and the removal of leaf primordia. Text-figs. 13, 14, and Pl. VI, Fig. 5, illustrate the protostelic condition of the stele in the



TEXT-FIGS. 7-14. Representative transverse sections from apex to base of a young rooted sporophyte plant from which leaf primordia have been removed (Pl. VI, Fig. 6). Text-fig. 7. Near distal region of shoot: undifferentiated shoot-stele on right, small leaf-trace on left. Text-figs. 8, 9. Stages in the progressive reduction of the stele. Text-figs. 10, 11. Approximately solenostelic vascular system present in the experimental region of the shoot. Text-fig. 12. Dictyostelic condition in the older region of the shoot, below the defoliated region; *Lt.*, the two vascular strands of a leaf-trace; an incipient inner vascular strand is present in the central pith. Text-figs. 13, 14. Protostelic condition near base of shoot: the protostele at the apex is of approximately the same diameter as the protostele. ( $\times 22$ .)



basal region of the shoot, i.e. near the point of attachment to the prothallus. This protostele compares closely in size with the protosteles observed below the apical meristem in the two experimental plants. Thus the progressive increase in stelar complexity which accompanies increase in size during the development of the individual (Bower, 1930) has been completely reversed as a result of continuous defoliation.

In this second plant starch was abundant in the lower region of the shoot. Starch grains in small numbers were also present in the cortex and pith in the solenstelic, defoliated region, but diminished near the apex.

In the lower part of the shoot the pericyclic tissue situated immediately outside the phloem consisted of conspicuous radial rows of cells. This cambiform tissue (Pl. VI, Fig. 9) showed no differentiation into vascular elements. On its outer limit the endodermis, as in the normal adult stele in *Angiopteris*, could not be distinguished. Evidence of a growth stimulus was also apparent in the metaxylem and xylem parenchyma where certain cells had developed to large size and had undergone divisions. Cambial activity in the Marattiaceae has already been mentioned by other investigators. In the plant under consideration the root system remained undisturbed and hence it is probable that some uptake of mineral nutrients was in progress throughout the period of the experiment. On the other hand, the active utilization of nutrients at the distal region of the shoot was precluded by the conditions of the experiment. The secondary growth activity in the lower region of the shoot may therefore be referable to the absence of active growth in the apical region. Another possibility is that wound hormones produced during the excision of successive leaf primordia may have moved basipetally and produced growth stimulation. In *Dryopteris aristata* comparable developments of the pericycle have also been observed in experimentally wounded specimens (Wardlaw, 1946b).

In a third experiment a young rooted plant which had arisen from a detached petiole-base was defoliated for some time. Later, when defoliation was discontinued, a group of small leaf primordia developed round the shoot apex. The effect of defoliation was twofold: firstly, the vascular system, which was dictyostelic below, became solenostelic in the experimental region; and secondly, the shoot became attenuated and the vascular system underwent a notable reduction in size and complexity (Pl. VI, Figs. 10, 11). Thus in the terminal leafy region there was a small protostele to which the small leaf-traces became conjoined. In the basal region a solenostele with a small central pith was present, the 'departure' of the leaf-traces being comparable with that in solenostelic ferns.

#### INITIAL DIFFERENTIATION OF VASCULAR TISSUE IN THE SHOOT

In the ferns as a whole, and in the Marattiaceae in particular, a special interest attaches to the interpretation of the vascular column of the leafy shoot. Thus, according to some investigators, the shoot stele is to be regarded as an essential constituent of the axis or shoot, whereas others have held that it is a com-

posite structure consisting of fused decurrent vascular strands from the leaves. Campbell (1921) has pointed out that in the Marattiaceae and other eusporangiate ferns there is apparently 'an absence of a cauline stele in the young sporophyte'. The young plant is therefore regarded by him as being composed of leaves as primary organs, the so-called stem being formed by the coalescence of leaf-bases. While very young sporophyte plants such as those described and illustrated by Campbell have not been examined by the writer, particular attention has been paid to the apical tissues of young sporophyte plants and young petiole buds. In actively growing plants which have been fixed and stained following the writer's usual procedure it has been possible to observe that the vascular tissue of the shoot originates immediately below the apical meristem as in leptosporangiate ferns (Wardlaw, 1943, 1945). In *Angiopteris* longitudinal sections are more suitable for this demonstration than transverse sections (Pl. VI, Fig. 12).

#### DISCUSSION

It has been shown that if young leaf primordia are excised from the shoot of *Angiopteris evecta*, leaf-gaps are not formed and the dictyostelic vascular system becomes solenostelic. This result, for one of the more primitive ferns, supports earlier findings regarding the influence of leaf development on the morphology of the shoot-stele (Wardlaw, 1944a, 1945, 1946). Although the main object of the investigation has been achieved, *Angiopteris evecta* is considerably less suitable for the kind of experiment undertaken than are species of *Dryopteris*. This is due partly to technical difficulties and partly to the nature of the material. The shoots of defoliated plants of *Angiopteris* have consistently become attenuated and have shown a rapid reduction in the size and complexity of the vascular system. In one plant, for example, a polycyclic, dictyostelic vascular system was reduced to a solid protostele; this protostele decreased in size and eventually faded out about 1 mm. below the distal region of the shoot.

The data obtained are consistent with the view that in *Angiopteris* the shoot stele is not merely composed of decurrent leaf-traces but that vascular tissue of axial origin is also present. It has been seen that the growth of leaf primordia affects not only the development of the vascular tissues, as previous investigators have maintained, but of all the tissues of the shoot. The former relationship is of particular interest in view of some observations recorded by Campbell (1911, 1921). In his view the stelar theory as usually understood (i.e. that the fibro-vascular skeleton of the fern shoot, with which the foliar-bundles or leaf-traces are connected, is of truly axial or cauline origin) 'cannot be reconciled with the facts as revealed by a study of the Eusporangiateae'. He considers that this view is supported by the investigations of Brebner (1896, 1901, 1902) and West (1917). He further states that from an extensive series of investigations on nearly all the genera of eusporangiate ferns he was forced to the conclusion 'that a cauline stele is either completely wanting in these ferns, or that, where cauline stelar tissues are present, they constitute

an insignificant part of the fibro-vascular skeleton'. And again he remarks: 'The "dictyostele" of *Ophioglossum* and most *Marattiales* is in no sense a monostele. The "foliar gaps" are not breaks in a single tubular stele, but are merely spaces between the coalescent leaf-traces, and the pith is part of the ground tissue included within the cylindrical network formed by the united bundles derived from the leaves.' He is, moreover, prepared to find that a close analysis of the vascular tissues of the young sporophytes of leptosporangiate ferns may well yield comparable results.

If these conclusions can be substantiated, they are clearly of great importance in considering morphological theories relating to the fundamental nature of the shoot. In particular, the phytonic view, i.e. that the shoot is composed of phytons or foliar segments which become united during growth, tends to be maintained not merely as a subjective idea but as an objective organographic view of shoot development. Campbell does in fact consider that his investigations of the young sporophytes of *Marattiales* and *Ophioglossales* to some extent afford 'a confirmation of Delphino's theory that the leaves, instead of being appendages of the stem, are the primary organs, and that the so-called stem is formed by the coalescence of leaf-bases'. Furthermore he maintains that the very young sporophytic plant of *Ophioglossum*—by general consent the most primitive of living ferns—has no stem at all, but consists merely of a single leaf and root, 'the stem arising secondarily as an adventitious bud'. . . . 'And the predominance of the leaf, shown in the young sporophyte, is maintained throughout the whole history of the *Filicineae*.' . . . 'The assumption, therefore that the stem is the predominant or primary organ of the sporophyte, and that the leaves are mere appendages of this, is hardly borne out by a study of the ontogeny, at least of the *Eusporangiateae*; and this probably will be shown also to be the case in many, at least, of the *Leptosporangiateae*.'

West (1917) notes that, whereas in the sporeling of *Danaea* the vascular system consists entirely of leaf- and root-traces, 'part, at least, of the vascular tissue of the "siphonostele" with large leaf-gaps which marks the next stage in the elaboration of the stelar system of *Danaea*, is made up of elements which have a truly cauline origin and serve to connect up adjacent leaf-traces'. In *Danaea* the inner or medullary vascular system which arises by the gradual elaboration of the central commissural strand passes through a series of elaborations 'strictly analogous to those of the original vascular cylinder'. This inner vascular tissue is, however, regarded as being of cauline origin. Although West has considered the cellular constitution of the apical meristem he has not dealt with the incipient vascular tissue, whether of leaf or shoot, in the growing region below the meristem. Blomquist (1922) states definitely that no cauline vascular tissue can be seen immediately below the shoot apex.

Thus whereas the cauline origin of the vascular system of the shoot in leptosporangiate ferns such as *Dryopteris* is no longer in dispute (Wardlaw, 1944a), it will be seen from the foregoing remarks that a comparable origin for the vascular system of eusporangiate ferns such as *Angiopteris* has been

more or less explicitly denied. Nevertheless, the solenosteles and dictyosteles of the two groups of ferns are broadly speaking similar in all major structural details. On general grounds it seems improbable that two utterly different systems of construction are involved, and West, at least, has indicated that some of the vascular tissue in the shoot-stele of *Danaea* is demonstrably of cauline origin. In the present writer's view the facts of stelar development and construction in eusporangiate and leptosporangiate ferns may be brought into harmony by the following considerations. The hypothesis has been advanced that wherever the apical meristem of a shoot, leaf, or root is in a state of active growth, of such a nature that the distinctive character of the meristematic cells is maintained, the initial differentiation of vascular tissue will be observable immediately below the apex and in the path of substances diffusing from it, one or more of these substances being causally involved in that process (Wardlaw, 1944). In leptosporangiate ferns the incipient vascular tissue can easily be observed, in suitably stained preparations, in actively growing apices: in comparable apices fixed during the quiescent period the vascular tissue may be considerably less evident or indeed not apparent. Similar evidence has also been obtained from a study of the renewed growth of isolated meristems or bud primordia (Wardlaw, 1943, 1943a, 1946a). Moreover, in attenuated plants of *Onoclea* it was shown that as the activity of the apical meristem diminished and finally ceased, the vascular tissue of the shoot faded out (Wardlaw, 1945a). These and related observations which need not be cited in detail indicate that the initial differentiation of vascular tissue is directly related to the activity of the apical meristem. The statement that no shoot apex is present in very young embryos of the Ophioglossales and Marattiales and that it appears later as an adventitious bud on the surface of one of the early leaves seems to the writer to require revision: what is evident is that the shoot meristem of the embryo is inconspicuous and relatively inactive. The writer's investigations of slightly older plants than those described by Campbell show clearly that an apical meristem is present and that incipient vascular tissue can be observed immediately below it. These facts, together with the observation that vascular tissue could be traced into the apical region of shoots from which all leaf primordia had been removed, leave little doubt that in origin and organization the vascular system in *Angiopteris evecta* is closely comparable with that of leptosporangiate ferns. In this view the leaf-gaps in *Angiopteris* originate in the same way as do those in *Dryopteris*. In *Angiopteris*, however, because of the active and extensive growth of the leaf and the relative inactivity of the shoot apex, the contribution of the leaf-traces to the shoot stele is pronounced. Moreover, in relation to the extensive development of leaf-gaps, incipient cauline vascular tissue is transformed into parenchyma as previously described (Wardlaw, 1944a, 1945).

A notable feature of these defoliation experiments has been the rapid attenuation of the shoot with concomitant reduction in the size and complexity of the vascular system. In fact, an almost complete reversal of the

ontogenetic developmental sequence has taken place. Such evidence indicates the limitations of the Theory of Recapitulation as an explanation of the progressive increase in structural complexity during the individual development: on the contrary it shows that the changes observed should be analysed in terms of the factors involved in growth. Evidence of the size-structure correlation (Bower, 1930) is afforded by the data obtained. The supply and interaction of metabolic substances in the growing region are among the factors which determine the actual size attained.

If the view be entertained that there is 'competition' between the developing leaf primordia and the shoot apex for the nutrients proceeding from below, it might perhaps have been anticipated that the removal of all leaf primordia would have been attended by an increase in the size of the shoot. Actually the apical meristem gradually disappears and the shoot undergoes a progressive decrease in diameter. This type of decrease—which has sometimes been described as a 'starvation' effect—is not due primarily to lack of carbohydrate. This is in agreement with the results of an earlier investigation of attenuated shoots of *Onoclea* (Wardlaw, 1945a). Inadequate supplies of nitrogen or mineral nutrients may also be excluded as the cause of attenuation since fully rooted plants have shown a diminution of apical growth comparable with that of feebly rooted specimens. The upward movement of solutes may, of course, have been affected if transpiration was curtailed as a result of the excision of leaves. Goebel (1905) has developed the conception of the apex as an 'attraction centre', i.e. that the more active apical regions draw on synthetic materials from the less active basal regions and use them during growth. It is conceivable that on the removal of the leaf primordia this general acropetal movement of nutrients is diminished.

During the normal development the growth of the apical meristem and of the tissues of the shoot is dependent on amino-acids, or, more generally, protein precursors and activating substances produced by developing and adult leaves. The supply of such substances would be eliminated or reduced by the experimental treatment. The influence of older leaf primordia on younger ones has been seen in experiments described above in which an apex was defoliated and then allowed to continue its development: the new leaves which developed were of small size and simple structure, like those of young sporophyte plants, and the vascular system of both shoot and leaves showed a great reduction in size and structural complexity.

The influence which developing leaf primordia exercise on the growth of younger primordia and on the shoot apex still awaits detailed investigation. Goodwin (1937) has shown that in the basal rosettes of *Solidago sempervirens* only one leaf is rapidly elongating at a particular time and that such a leaf retards the development of the younger leaves. The removal of the rapidly growing leaf is attended by the elongation of the next succeeding leaves. As the diffusion of auxin was greater from rapidly growing leaves than from leaves at any other stage, Goodwin considers that the retardation of younger by older leaves was probably due to a surplus production of auxin by the latter.

He has also demonstrated that the total amount of auxin diffusing from a young leaf primordium is very small but increases to a maximum during the most rapid period of growth. Albaum (1938) in an investigation of growth hormones in fern prothalli and sporophytes has shown that the auxin-producing centre of the young sporophyte is the primary leaf. This leaf produces a substance which inhibits the outgrowth of adventitious processes in the prothalli and also the development of other leaves. If the findings of Goodwin and Albaum are applied to the experimental materials of *Angiopteris* it might be anticipated that the removal of leaf primordia would be attended by more rapid and more extensive growth of the shoot. This is not the case.

As against the findings of Goodwin and Albaum, Snow and Snow (1937) have observed that when heteroauxin-in-lanoline paste was applied to shoot apices of *Lupinus Albus* and *Epilobium hirsutum* there was a very considerable enlargement of the leaves and axillary buds in the region of application. Moreover, as Snow (1937), Thimann and Skoog (1934), and others have shown, activating substances produced by young growing leaves promote the growth of the shoot below. In *Angiopteris* also, it may be that the growth of the apical meristem and of the developing shoot is stimulated by substances proceeding from the developing leaf primordia: on the excision of primordia these substances will no longer be supplied and attenuation will ensue. There is the further consideration that the removal of leaf primordia makes for the appearance of new primordia and hence, if these are consistently removed, a depletion of substances necessary for meristematic activity and growth may result. It may be noted here that after a period of continuous defoliation a stage is reached when the apical meristem is more or less completely transformed into parenchymatous tissue. Comparable changes have already been described and illustrated by Holloway (1939) for vasculated prothalli of *Psilotum triquetrum*.

An impressive feature of the individual development from the embryo (or bud primordium) to the adult is the orderly manner in which one phase of development succeeds another, with concomitant increase in structural complexity; and how, irrespective of the actual size of the apical meristem, the phyllotaxis remains constant. In experimental plants undergoing a progressive decrease in size this same orderliness has also been evident. Such observations indicate the importance and constancy of reaction of the hereditary substance. Elsewhere the writer has pointed out that the histological character of the apical region remains constant throughout the individual development, this being in marked contrast to the diversity of tissue pattern to be observed at different levels in the shoot. In other words, whereas the nature of the physiological activity at the apical meristem tends to remain constant throughout development, very marked changes take place in the growth and differentiation of the tissues below the apex at different stages during the individual development. Such reasoning points to the importance, in the interests of morphological study, of an analysis of the factors involved in the development of the growing region. Only by such an approach

will it be possible to separate extrinsic morphogenetic factors from those which are intrinsic, i.e. truly inherent in, and pertaining to, the genetical constitution.

### SUMMARY

On the destruction of a succession of leaf primordia in *Angiopteris evecta* the normal dictyostelic shoot becomes solenostelic and eventually protostelic, the attenuation of the shoot being a feature of such experiments.

The results obtained support the view that the normal shoot stele is a composite structure including vascular tissue of both axial and foliar origin. In relation to the active growth of the leaves and the relative inactivity of the shoot apex, the foliar contribution to the shoot stele tends to be pronounced.

The results obtained are consistent with the view that there is no fundamental difference in origin and organization between the steles of eusporangiate and leptosporangiate ferns. The phytonic view of the constitution of the axis in the former is opposed by the results of this investigation.

The attenuation of the shoot consequent on the removal of leaf primordia is discussed.

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## EXPLANATIONS OF PLATE VI

Illustrating Professor C. W. Wardlaw's article on *Angiopteris evecta*

All figures are from untouched photographs.

Fig. 1. Large bud dissected from old plant, rooted in peat, and systematically defoliated. The shoot, which has undergone a great reduction in diameter, is terminated by a small apical cone. ( $\times 1$ .)

Fig. 2. Transverse section of distal region of specimen illustrated in Fig. 1, showing an undifferentiated protostele; above this level the vascular tissue fades out and only parenchyma is present. ( $\times 80$ .)

Fig. 3. Section of same shoot lower down, showing a protostele with a solid core of tracheides. ( $\times 80$ .)

Fig. 4. Still lower down: two conjoined vascular strands. ( $\times 80$ .)

Fig. 5. Protostele at base of young sporophyte for comparison with Fig. 3. ( $\times 80$ .)

Fig. 6. Young rooted sporophyte plant from which leaf primordia were consistently removed. ( $\times 1$ .)

Figs. 7, 8. These transverse sections of the above specimen illustrate the approximately solenostelic condition of the vascular system consequent on the removal of leaf primordia in the experimental region of the shoot. ( $\times 25$ .)

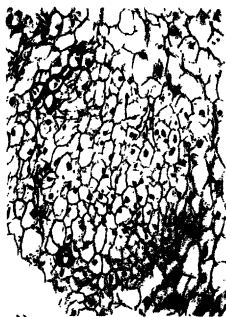
Fig. 9. Lower down in the same shoot: a cambiform development has taken place in the pericyclic region; there is also evidence of growth stimulation in the peripheral tracheides and vascular parenchyma. ( $\times 40$ .)

Fig. 10. Transverse sections of a petiole bud, showing the relation of a leaf-trace to the solenostelic shoot stele. ( $\times 60$ .)

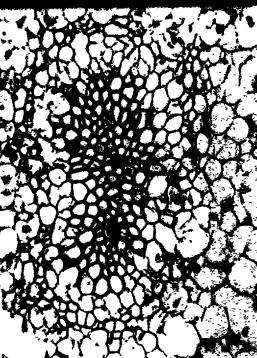
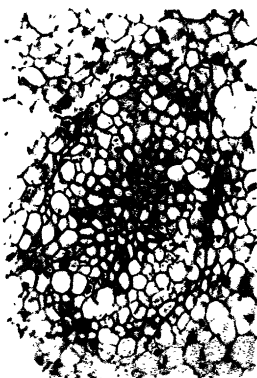
Fig. 11. Transverse section of the same specimen higher up, showing the great reduction in stelar size consequent on the removal of leaf primordia. Shoot stele, right; leaf-trace, left. ( $\times 60$ .)

Fig. 12. Longitudinal median section of a young sporophyte plant, showing the apical meristem and incipient vascular tissue immediately below. ( $\times 250$ .)

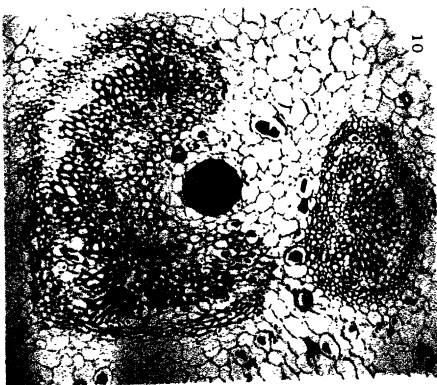




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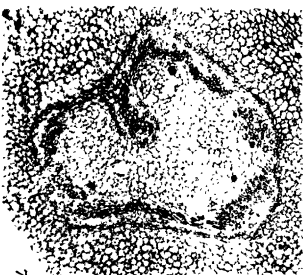
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Huth coll.





# On the Seedling of *Oxalis hirta* L.

BY

A. J. DAVEY

(From the Department of Botany, University College of N. Wales, Bangor)

With nine Figures in the Text

**D**URING the course of investigations on root contraction in species of *Oxalis* (Thoday, 1926; Thoday and Davey, 1932) reference was made to the work of Freidrich Hildebrand (1884) on the life histories of certain species of this genus. It included a description of the seedling of *Oxalis rubella* Jacq. with its remarkable method of sinking the first bulb (formed from the plumular bud usually after one foliage leaf has expanded) from the soil surface to a depth of several centimetres. Curiosity was aroused regarding the method of sinking, for according to Hildebrand's description it differs radically from the root contraction which is responsible for the sinking of bulbs in so many other species of *Oxalis*; moreover, the seedling root of *O. rubella* shows no external signs of any contraction. In addition to *O. rubella* Jacq., Hildebrand referred to a number of other species (obtained by him from Kew and Palermo) closely resembling it in adult and seedling morphology as well as in the method of germination and the sinking of the first bulb. These species included *O. hirta* L., *O. multiflora* Jacq., *O. fulgida* Lindl., *O. tubiflora* Jacq., *O. canescens* Jacq., *O. macrostylis* Jacq., and *O. longisepala* Tod.<sup>1</sup>

The adult plants of these species produce no contractile roots and have no means of lowering or otherwise altering the position of their bulbs. However, in another group of closely allied species which included *O. flava* L., *O. Coppolieri* Tod., and *O. fabifolia* Jacq., Hildebrand (1884) found that not only in seedlings (*O. Coppolieri* Tod., loc. cit., Pl. V, Figs. 18–20), but also in adult plants the lowering of bulbs was brought about by a method essentially similar to that obtaining in the seedling of *O. rubella*. (loc. cit., pp. 70, 71, 74, and 76, Pl. I, Figs. 3–7).

In 1888 Hildebrand published the results of further studies on *Oxalis rubella* amplifying his former account and suggesting an explanation of the

<sup>1</sup> Note on specific names. Salter in his recent monograph (1944) considers *O. hirta* L. to be a group species under which he includes, among others *O. multiflora* Jacq., *O. macrostylis* Jacq., *O. rubella* Jacq., and the other species referred to by Hildebrand. *O. hirta* L. is a member of section J: Crassulae (caulescent forms). Similarly, *O. fabaefolia* Jacq. is held to include *O. Coppolieri* Tod., *O. asinina* Jacq., *O. lancaefolia* Jacq., and *O. leporina* Jacq. *O. flava* L., includes *O. flabellifolia* Jacq. *O. flava* and *O. flabellifolia* are classed in Section J: Angustatae (stemless forms). Salter does not consider *O. Consolei* Tod., *O. Majeranae* Tod., and *O. Coppolieri* Tod. to be worthy of distinction. In correspondence with the author in 1938 he spoke of the 'so-called species *asinina* and *fabaefolia*' as 'indeterminate interlocking groups almost impossible to be separated from *O. flava* (= *O. flabellifolia* Jacq.) on account of intermediates'.

descent of the plumular bud. Fig. 1 is reproduced from his article. All Hildebrand's descriptions are in the main morphological and take little note of the detailed structure and behaviour of the root, which is assumed to play no active part in the lowering of bulbs.

Since 1888 no further investigations on seedlings of *O. rubella* or its close allies can be traced. Hildebrand's description is briefly cited by Reiche in his account of the Oxalidaceae in Engler's 'Pflanzenfamilien' (1897). Some of

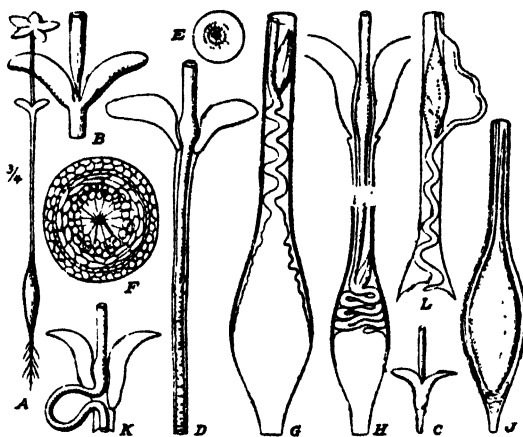


FIG. 1. Stages in development of *Oxalis rubella*. A. Seedling with water-storage root, cotyledons and 5-membered foliage leaf. B. Longitudinal section through cotyledons and growing-point. C. Cotyledonary sheath. D. Growing-point travels down due to extension of petiole downwards. E. Transverse section young root. F. Transverse section through water-storing root. G. Formation of young bulb from growing-point and resorption of 'water storer'. H. Young bulb has reached 'water storer' which is half resorbed. J. 'Water storer' completely resorbed: tips of bulb scales extend into root tube: stalk of foliage leaf to left. K. Cotyledonary sheath broken through, due to its weak resistance to early thickening of bulb: leaf-petiole is extruded and bulb cannot sink deeper. (Figures and legend after Hildebrand, 1888.)

Hildebrand's figures, together with his explanatory annotations, appear in R. Knuth's account of Oxalidaceae in 'Pflanzenfamilien' (1931) and again in the section on Oxalidaceae by the same author in 'Das Pflanzenreich'. The same set of figures (Fig. 1) is reproduced by Troll in his 'Vergleichende Morphologie der höheren Pflanzen', 1937. Troll summarizes Hildebrand's account of the seedling and remarks that 'ein umfassenderes Studium dieser einzigartigen Verhältnisse steht leider immer noch aus'. The present paper attempts to remedy this deficiency.

The work has been long delayed owing to exceptional difficulties in obtaining seed. Viable seed could not be obtained from overseas because the seeds retain their power of germination for only a few hours after shedding, and cannot be dried without injury. On the other hand, for some years all plants obtained from whatever source were long styled and self-sterile, so that seed could not be obtained from home-grown plants.

The first seedlings examined were three dried specimens kindly sent in 1939 by Captain Salter, R.N., from S. Africa in response to the author's

inquiries. Soaking followed by clearing revealed the main features of the contractile process. The preparations, with photographs, were exhibited, together with illustrations of root contraction in other species of *Oxalis*, at the British Association meeting in Dundee in 1939.

The plant now under consideration has been identified by Mr. Exell of the British Museum as *Oxalis hirta* L. This is listed in Index Kewensis as *O. hirta* L. (= *O. rubella* Jacq.); *O. rubella* Jacq. is the name used by Hildebrand. He refers to *O. hirta* Linn. as differing in certain details from *O. rubella* Jacq. Plants grown here from bulbs obtained from various sources in this country agree closely with *O. hirta* sent from S. Africa by Captain Salter. Certain differences in details of seedling structure between *O. hirta* and Hildebrand's plants, which will be noted later, make it somewhat doubtful whether his '*O. rubella* Jacq.' was identical with *O. hirta* Linn. Attempts to trace herbarium specimens or living plants were interrupted by the outbreak of war.

During the preparation of this paper for the press Salter's monograph on the genus *Oxalis* in S. Africa has come to hand. He considers *O. hirta* L. to be a group species consisting of numerous forms and varieties among which are included the related species and varieties mentioned by Hildebrand. *O. rubella* Jacq. is listed as *O. hirta* L. *a typica*, form E.

In *O. hirta* as in most other species, the seeds are shed explosively by the sudden inversion of the outer seed-coat. This makes collection in the field difficult. In contradistinction to many other species, the seeds of *O. hirta* and its near allies have no period of rest or dormancy between shedding and germination. Seeds do not retain their viability if allowed to dry. A few seeds sent by air mail from S. Africa in 1938 through the courtesy of the directors of Hocker Edge Gardens had already withered before they arrived.

In common with many other species of *Oxalis*, *O. hirta* has trimorphic flowers, producing long-, medium-, and short-styled flowers exclusively on different individuals. These are completely self-sterile, and artificial cross-pollination between individuals of the same style length is ineffective. Seed is set only when 'legitimate' pollination takes place between individuals of different style length, presumably by the agency of pollen from the stamens of length corresponding to that of the style.

During a number of years all plants obtained from whatever source bore long-styled flowers and were self-sterile, and sterile also when cross-pollination was effected between different individuals, so that no seed could be obtained. It is probable that all plants at present under cultivation in this country are long styled. The plants multiply rapidly by means of bulbs or bulbils produced in the axils of the scales of the parent bulb and from buds in the axils of scale leaves on parts of the stem which are below ground.

In 1939 a collection of bulbs was received from Captain Salter, R.N., from the National Botanic Garden, Kirstenbosch. From these bulbs there were raised 30 plants, of which 3 proved to be short styled and 2 medium styled the remaining 25 were long styled and similar in all details to the long-styled

plants already obtained from various sources in England. Seed was first obtained from these plants in the autumn of 1940. A small stock of the three forms has since been built up which seeds readily when pollination between flowers differing in style length is carried out by hand. Natural pollination requires the agency of insects which are absent from the greenhouse in which the plants were grown. Plants were removed to a light chamber for pollination when continued lack of sunshine kept the flowers closed. The corollas unfold readily when exposed to the light of a 500-watt Mazda lamp. (Hildebrand was fortunate in that his collection included forms and varieties of different style length which were freely visited and pollinated by bees.) Under cultivation here the long-styled plants are more vigorous and prolific than either of the other two forms, and selection for vigour may well account for the absence of the latter from horticultural stocks and also perhaps for the small proportion of them among the bulbs sent by Captain Salter. The bulbs are planted in late August. They grow rapidly and flower in late September and throughout October. Seed ripens from late November to mid-December. After flowering, vegetative growth and branching becomes slower and finally comes to a standstill while the new bulbs are developing and ripening. By April the leaves begin to change colour and wither. The plants are now allowed to dry off and complete the ripening of their bulbs. As noted by Hildebrand, the bulbs begin to sprout in late August even if not planted.

#### *Germination and growth of seedling.*

This agrees in general with Hildebrand's description, though there are small differences in structural details. When the seed is shed, the embryo is covered only by the thin translucent inner seed-coat. Seeds germinate readily on moist soil or sand. The deep green embryo consists mainly of its two fleshy cotyledons which are packed with starch. The radicle is extremely short and is completely covered by closely set downward-growing mucilage-secreting hairs derived from the epidermis of the very short hypocotyl. They are single-celled outgrowths of superficial cells comparable with root-hairs. The first plumular leaf is well advanced and green, consisting of a short petiole bearing five ovate leaflets, longitudinally folded. The vascular system is correspondingly advanced in development and shows lignification of vessels in the cotyledons, the base of the first foliage leaf and the hypocotyl.

Within a few hours of placing in moist conditions the cotyledons swell and diverge from one another, breaking out of the thin coat. The petiole of the first plumular leaf begins to lengthen, carrying the folded leaflets beyond and above the cotyledons. The second leaf remains for some time very small and rudimentary. The cotyledons are united at their bases, forming a short tube which attains its final length of about 2 to 3 mm. at an early stage. The interior of the tube is funnel-shaped and closely surrounds the enclosed basal portion of the plumular petiole, which fits it like a plug with the thickest part uppermost.

In contact with the moist soil the secretory hairs produce a jelly-like drop of mucilage which surrounds the root-tip in its early stages of growth until it has penetrated the soil. The root for a time grows vertically downwards without branching; its surface bears many short root-hairs. As was remarked by Hildebrand, the root is able to bore straight through hard clay lumps by which the roots of other plants are diverted from the vertical. The hypocotyl region bearing the mucilage hairs does not lengthen at all and may be recognized at all stages in the seedling development by the shortness of its cells and the remains of the mucilage hairs. The first plumular leaf usually has 5 leaflets, but the number may vary from 4 to 7. At first each leaflet is longitudinally folded and all are bent downward, but they soon expand and spread out horizontally. The lengthening petiole carries them up to a height averaging about 3 cm. above the soil. Growth in length of the petiole above ground is complete within 4 or 5 weeks of germination. The leaflets continue to enlarge for some time after this and may reach a length of over 1 cm. Usually only one foliage leaf is produced in the first season, but exceptions occur in which the second leaf develops similarly to the first; like all succeeding foliage leaves it has only three leaflets.

The root grows downwards without branching until it is from 4 to 9 cm. long. At this depth there occurs a gradual swelling of a short limited region which tapers gradually above and passes over abruptly into a thin slender branching absorptive part below (Figs. 2 and 3). The onset of the swelling seems to be correlated with slowing of the elongation of the main root. Branching is mostly confined to the region below the swelling. (Among the many seedlings examined only one produced a branch root above the swelling; a very few showed a branch arising from the swollen region.) The swelling increases till at its widest part it may be three or four times the diameter of the upper part of the root, which by that time is brown and slightly shrunken. The distance from the soil surface to the base of the swelling varies between 3.5 and 8 cm. approximately (average 5–6 cm.).

Seedlings were grown in pots and also in wooden observation boxes provided with one glass side darkened by means of a close-fitting metal shield. There was remarkable uniformity in the growth and behaviour of most of the seedlings, but the roots of those grown against the glass in the observation boxes reached a greater length and were slower in completing their development than those grown in pots or in the part of the boxes away from the glass. The depths at which the swelling occurs and to which bulbs were lowered averaged 4–6 cm. for those in pots and 11–12 cm. for those in boxes against glass. The explanation of this difference must await further experiment. Finally, by the end of March or early in April the swollen part of the root contains a small bulb whose enlarged base usually splits the root tissues covering it (Figs. 2 and 3). The leaflets are yellowing and beginning to wither; the cotyledons are shrivelled and may have fallen, though they often persist in a shrunken condition for a considerable time. Outwardly there is no sign of the process by which the bulb has travelled from its original position at the



cotyledonary node to depths varying from 3 to 12 cm. below the soil surface. The bulb ripens and turns brown; and the root and first leaf-petiole above ground persists in a withered condition for some time, often until next season.

Investigation shows that above the ripening bulb the root now contains a smooth white thread which is an interpolated part of the petiole of the foliage

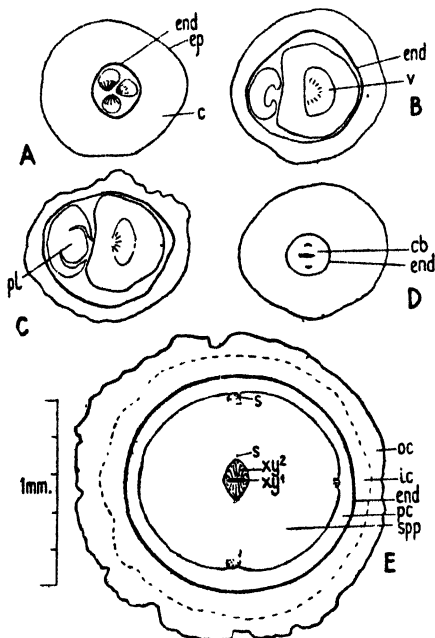


FIG. 2.

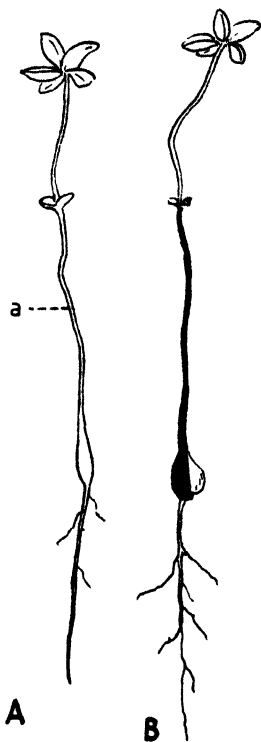


FIG. 3.

FIGS. 2-3. Fig. 2. Outline T.S. of seedling at different levels after contraction has set in. A, Aerial part of petiole; B, root tube enclosing petiole and second leaf; C, plumular node; D, root of younger seedling for comparison; E, swollen part of root. end, endodermis; oc, outer cortex; ic, inner cortex; cb, cambium; v, vascular strand; spp, secondary phloem parenchyma; pc, pericycle; s, sieve tubes area; xy<sup>2</sup>, secondary xylem; xy<sup>1</sup>, primary xylem; pl, plumular axis. Fig. 3. *O. hirta*. A. Seedling at time when contraction is taking place. (a shows position of bud.) B. Seedling at end of season; ripening bulb protruding from base of tube  $\times \frac{3}{4}$ .

leaf; above, this is firmly gripped by the cotyledonary tube; below, it is still attached to the plumular node at the base of the bulb. Attached to the base of the bulb is a tightly coiled and compressed thread, which is the core of the root which formerly occupied the place now filled by the petiole. Thus the apparent root above the bulb is now merely a tube formed from its outer tissues.

At an early stage the inner surface of the cotyledonary tube is funnel-shaped, sometimes slightly contracted towards its upper end, but never so markedly as in Hildebrand's figures of *O. rubella*. The enclosed part of the

petiole accommodates itself to the form of the cotyledonary tube which fits it tightly. The exposed green surface of the petiole and leaflets is clothed with stiff hairs. The basal region of the petiole within the tube is pale coloured and completely devoid of hairs, and is meristematic. The renewed growth of this intercalary region is responsible for the downward extension of the petiole into the root tube.

Above the cotyledons the petiole is approximately circular in cross-section, but in its basal region the abaxial side is flattened or incurved. It receives a slender vascular strand from each of the five leaflets, but these immediately become closely aggregated into a central group (Fig. 2). (In *O. rubella*, according to Hildebrand (1888), the five strands retain their separate identity and are widely spaced throughout the petiole and its base (Fig. 4).) The cotyledons are traversed by slender strands which unite to form a pair of bundles in the base of each, where rearrangements take place in the shortest possible vertical distance in transition to a diarch root structure (the root may distally become triarch or tetrarch). In other words, transition from stem to root structure occurs at a very high level. At the cotyledonary node the strands from the two plumular leaves, situated in the intercotyledonary plane, are composed mainly of secondary elements. Secondary thickening proceeds so that the very small diarch xylem plate of the primary root is flanked by two wedge-shaped masses of secondary xylem. Finally this xylem may form an oblong mass or plate in the plane at right angles to the primary plate. The vessels are spiral with some transitions to loosely reticulate forms.

Most of the secondary phloem consists of parenchyma, which forms a wide zone round the small xylem strand with the two first-formed groups of sieve tubes at its outer circumference. The secondary phloem parenchyma contains no air-spaces; its cells possess a nucleus embedded in a thin film of cytoplasm, and a large vacuole containing watery sap in which reducing sugars occur. Starch is never found in the root, but it abounds in the large-celled cortex of the petiole. The sieve-tube groups consist of sieve tubes with companion cells associated with secretory cells containing a resinous oily substance. These latter are found also in association with the xylem, in the pith at the node, and in connexion with the vascular tissues throughout the seedling. Surrounding the phloem is a pericycle and a well-differentiated endodermis, showing a Casparian strip but later having the whole wall suberized. This endodermis is clearly defined throughout the short hypocotyl as far as the cotyledonary node. All the tissues are very similar to those found in the much larger fleshy contractile roots of *O. incarnata*, *O. cernua*, and many others.

Increase in diameter due to cambial activity is not great and is rendered less apparent in the upper part of the root by the shrinking of the root cortex consequent on the early development of periderm. In the swollen part of the root down below, increase in diameter is chiefly due to enlargement of the constituent cells of the phloem parenchyma, which are frequently binucleate. The swelling takes place prior to periderm formation in this region, and there

is a correlated increase in size of the cortical and endodermal cells. These latter are greatly stretched tangentially, but counts of cells at this level and in the narrower part above showed no significant difference in their number.

Periderm arises by the tangential division of the layer of pericycle immediately abutting on the endodermis; this produces one or two layers of cells, the outermost becoming the first layer of cork. This process begins just below the cotyledonary node and proceeds downwards. The process of tangential division also extends upwards into the pericyclic region of the node, where it forms an absciss layer which will later cut off the node from the cotyledonary tube. The cork layer then begins to split away from the endodermis, while the root core contracts slightly in diameter, thus leaving a space between it and the surrounding endodermal tube. At first the space is scarcely noticeable and, in the early stages of contraction and descent, friction with the tube will not be negligible. (In fixed material cut by the microtome, the necessary processes entail shrinkage, so far unavoidable, which exaggerates the size of the space between the core and the tube.) At the same time the core begins to contract longitudinally. The first layer of cork cells persists as a continuous covering to the 'core'. The cells of the root cortex lose their contents and their walls become brown, collapsed, and crumpled, thus diminishing slightly the diameter of the root as a whole. As the separation proceeds downwards from the node the contracting root core comes to lie in a rigid tube consisting of the root cortex lined by the endodermis, the cells of which retain their shape. The first sign of splitting occurs just below the node in the short hypocotyl where the endodermis is fully and characteristically differentiated. At this level and thereafter downwards the separation leaves clean, smooth surfaces indicating that the split is produced by dissolution of the middle lamella between endodermis and cork. The separation is continued almost immediately upwards into the short nodal region, but here the absciss layers may be torn irregularly and the resulting surface of the node and of the surrounding cotyledonary tube may show broken parts of cell walls. It is as though the separation here were brought about by forcible pulling and tearing. This suggests that contraction of the root core initiates the process, separating the core from the endodermal tube and pulling the nodal region downwards away from the cotyledonary tube, as indicated by the broken cells.

#### *Details of the lowering process.*

Three processes may be distinguished which take part in the shortening of the root core.

1. *Growth contraction.* Most seedlings show evidence of a small amount of growth contraction, i.e shortening of cells of the contractile tissue (phloem parenchyma) while active growth is still in progress in tranverse directions (Thoday and Davey, 1932; Gravis 1926). This process can be effective only so long as the contractile tissue remains a continuous cylinder of living cells. The xylem core remains straight. Evidence of the contraction is seen in fine

transverse wrinkling of the longitudinal walls of the periderm, endodermis, xylem parenchyma, secretory cells, and vessels.

2. *Cell-collapse contraction.* This is much more obvious than the above and is responsible for considerable shortening of the root core. It proceeds on the same lines as has been described for *O. incarnata* (Thoday, 1926). In the phloem parenchyma, which is a very regular and uniform tissue, horizontal plates of cells previously undifferentiated from their fellows lose their cytoplasm and nucleus and then become depleted of their watery sap; this brings about a collapse of the cells. In any one layer the process proceeds from the periphery towards the cambium and the process of depletion and collapse spreads from one layer to another. Alternating with bands of collapsing cells there remain plates of living turgid cells retaining their protoplasm and sap. A characteristic appearance is often that of bands of at least three layers of collapsing cells separated by single layers of turgid cells, the central layer of the empty cells having been the first to collapse. The vertical walls of the collapsing cells fold or crumple so that the horizontal walls come to lie together; some crumpling is apparent in the horizontal walls also. This accounts for part of the diminution in the diameter of the root core. The collapsing process appears to follow a spiral course round the root; the spiral is most easily observed by noting the course of the turgid plates of cells in lightly stained, cleared preparations of the unsectioned core. The beginning of collapse may be coincident with or may follow closely upon the separation of the core from the endodermis.

As collapse proceeds, the plumular node, separated from the cotyledonary tube, travels down the tube formed by the root cortex with its endodermal lining. Correlated with this and facilitating it, there is renewed activity of the meristematic basal region of the petiole resulting in its downward elongation. As in *O. incarnata* and several other species, the contracting collapsing layers do not remain horizontal but become inclined upwards. As collapse proceeds this inclination may become very steep and then produces considerable reduction in the diameter of the root core. It is no doubt caused by friction between the descending core and the tube surface as in *O. incarnata*, where the friction is between the root surface and the surrounding soil (Thoday and Davey, 1932). Owing to collapse of the contractile parenchyma, the periderm is thrown into folds or wrinkles; the phloem strands are buckled and contorted, but remain alive and functional; and the xylem strand with its surrounding cambium is bent and contorted into a spiral which is often remarkably regular (Figs. 5 and 7). The sequence of processes continues downwards until it extends into the thickened region of the root. Collapse of the sloping layers becomes so complete that at the outward surface of each turn of the xylem spiral there remains nothing between the cambium and the periderm

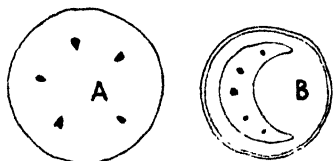


FIG. 4. *Oxalis rubella* Jacq. Outline T.S. aerial part of petiole (A) and of petiole within the tube (B). (After Hildebrand, 1888.) Compare Fig. 2 A and B.

but the closely adpressed walls of crushed parenchyma. The final form suggests either a twisted cord or a puckered, twisted ribbon, according as the xylem core is square or flattened.

3. *Spiral coiling of the root core.* Before the collapse-contraction has extended to the lower part of the root there is superimposed upon it a further shortening process, the coiling of the narrowed core *as a whole* into a spiral (Figs. 6 and 7). It begins in the upper part of the root immediately below the plumular bud and proceeds downwards, gradually winding the root core into

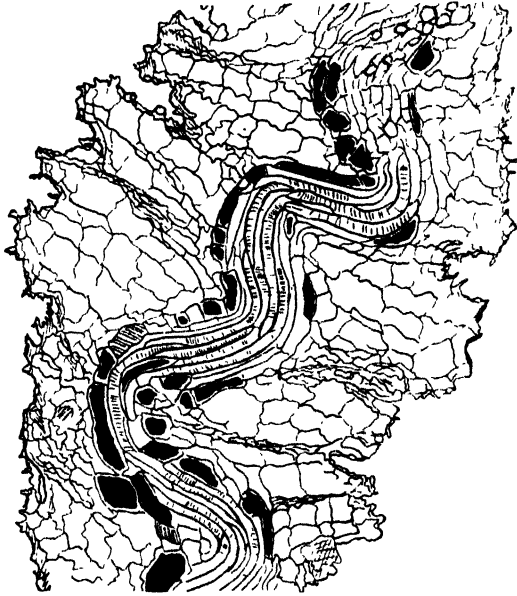


FIG. 5. *O. hirta*. Part of L.S. of contracted root core, showing arrangement of turgid and collapsed cells, and contorted xylem core. Secretory cells with black contents. (From a photograph.)  $\times 63$ .

a spiral, which becomes tighter until the turns of the coil are close together. One or more kinks caused by reversal of the direction of the coiling may occur. Throughout the coiling process the cambium remains active, adding elements to the xylem and phloem. As a result of the coiling the descending bud usually reaches the root swelling at a time when cell-collapse is beginning in the core of the swelling; by this time the bud has increased appreciably in size. As the bulb sinks lower into the widening tube of the swelling there is usually some relaxation of the coils, resulting in a straightening of the portion immediately below the bulb (Fig. 8). As the bulb continues to sink, the uppermost portion of the relaxing coil is displaced, so as to occupy a position between the bulb and the wall of the tube. Here it is finally held in position, pressed against the tube by the bulb, which expands so as to completely fill the available space.

The relaxation of part of the coil suggests that it may have been previously

under compression from the growing petiole, and raises the question of the mechanism of coiling and the part played by the petiole. Resistance of the coil to compression would become effective if the pressure on it were reduced, for example, owing to cessation of growth of the petiole. The expanding bulb is asymmetric in shape, swelling most on the side where the slit appears,

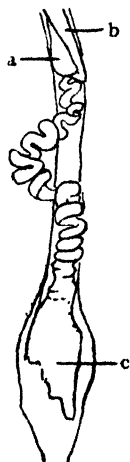


FIG. 6.

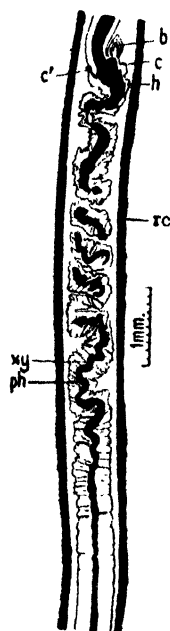


FIG. 7.

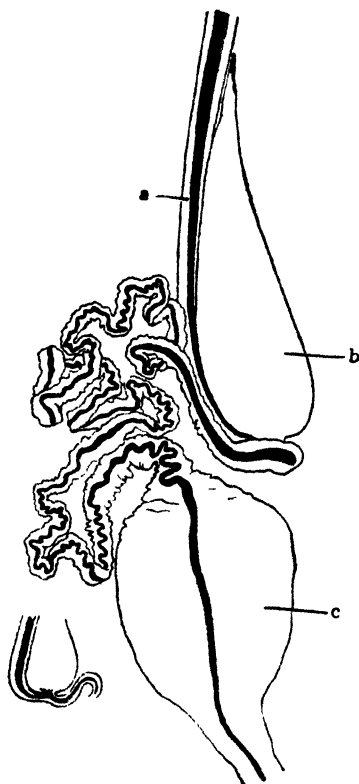


FIG. 8.

FIGS. 6-8. Fig. 6. *O. hirta*. Seedling with tube opened to show coiled core (displaced in handling). Fig. 7. Longitudinal section of root showing early stages of contraction and coiling of core. Zones of collapsed cells indicated by lines. *p*, petiole base; *h*, hypocotyl; *b*, apical bud; *c*, *c'*, remains of cotyledons; *xy*, xylem; *ph*, phloem parenchyma; *rc*, root cortex. Fig. 8. Camera lucida drawing of a cleared preparation; contraction nearly complete. Shows relaxation and straightening of upper part of core; rest of core (previously tightly packed above swelling) has uncoiled and stretched under treatment. Inset: vascular connexion to bulb. Vascular strands black. Fig. 6  $\times 3$ ; Fig. 8  $\times 10$ . Figs. 6 and 8: *a*, bulb; *b*, petiole base; *c*, root swelling.

through which the whole or greater part of it is finally extruded; the root coil is always retained within the tube partly below the bulb and partly on the side away from the slit. No instance of extrusion of the coil has been observed. There was no instance of further relaxation or uncoiling seen in the numerous examples which were opened up for observation. The coil can be stretched by hand almost to the original length of tube occupied by it previous to contraction and coiling. It is obvious that the xylem and phloem

remain functional in spite of great contortion, since the foliage leaf continues alive and the lower part of the root continues growth and branching.

Meristematic activity in a short basal region of the first leaf-petiole and considerable lengthening of the cells so produced result in intercalary extension of the petiole, which keeps pace with the contraction of the root core. The tight grip of the withering and shrinking cotyledonary tube holds in a fixed position the part of the petiole enclosed by it. No instance has been seen of any drawing down of the part above the ground.

There is some appearance of slight twisting of the petiole in its downward growth, such as the oblique course of the edges of the flattened adaxial face and of the elongated superficial cells; but nothing to indicate that the many spiral turns of the root core are continued in the petiole.

During its gradual descent the plumular bud becomes differentiated. The three leaflet rudiments of the second leaf are arrested in development, while the leaf base enlarges to form a sheath surrounding the rest of the bud; the third leaf develops similarly. Thus there are formed two thin protective bulb scales which become brown on ripening. Within these are differentiated usually three more leaves whose bases become fleshy scales, in which carbohydrate withdrawn from the foliage leaf and petiole is accumulated as starch. Slight enlargement takes place within the tube; further enlargement with considerable swelling of the storage scales takes place after the bulb has reached the root swelling. Here for some time starch continues to be transferred to it while fluid is supplied by the absorptive root system below. The ripe bulb, which is asymmetrical in shape, usually splits the root tube and its larger lower part protrudes wholly or in part outside it. The elongated scale-tips remain in the tube; no further change of level occurs. By this time the enclosed part of the petiole is shrunken and emptied of starch and the parts above ground are withered; the coiled core also becomes dried out. In the next season a slender short axis emerges from the tip of the bulb, still held within the root tube, and grows up through this to the soil surface, producing a leafy shoot resembling that of the adult plant; thus there is a sharp contrast between the first and all the shoots of succeeding years. In the first season there is produced a short shoot with one (occasionally two) petiolate 'radical' leaves. This condition is paralleled by the permanent adult state of many 'stemless' *Oxalis* species, e.g. *O. lobata*, *O. brasiliensis*, *O. flava*. In all subsequent seasons the shoots are 'long' shoots with extended internodes and leaves which lack an elongated petiole (sessile or subsessile).

#### *Departures from the normal course of development.*

Variations in behaviour were noted in 26 out of 143 seedlings examined when approaching maturity in March 1945. Of the 26, 1 had three cotyledons and 1 an abnormal number of leaflets. The remaining 24 showed abnormalities of growth or contraction as detailed below.

(1) In a number of seedlings, after the usual pause in growth of the petiole, meristematic activity of its base produced intercalary lengthening upwards,

pushing up the broadened or slightly swollen base of the first-formed aerial part out of the cotyledonary tube. The extension remained more slender and paler than the part above, from which it was sharply distinguished by its smooth, hairless surface. It varied from 3 mm. to about 3 cm. in length, and became slightly thicker than the meristematic region at the node and was therefore unable to slip or be drawn down the tube. It was less rigid than the first-formed upper region, and this caused the petiole to bend over or lie on the soil. In all such seedlings examined, upward growth of the petiole ceased after a time and downward elongation into the tube took place, as usual, correlated with lowering of the bulb.

(2) A less frequent variation (noted also by Hildebrand) is the occurrence of two foliage leaves. The second plumular leaf develops similarly to the first but with only three leaflets. The two petioles lengthen downwards into the tube at the same rate, closely pressed together, sometimes slightly twisted. Root contraction occurs and the bulb (symmetrically placed between the two leaf bases) is lowered as in normal seedlings.

(3) In some seedlings (4 only out of those examined in 1945) the bud emerged laterally from the tube after it had descended part way but was still very small, probably through weakness of, or injury to, the root tube. The bulb remained in a stationary position close to the outer surface of the tube. There was no emergence of either petiole or root core. The core showed evidence of collapse-contraction, which was still proceeding actively near the swollen part of the root where alternate layers of empty and turgid cells were obvious. The upper part of the core, instead of being wound spirally, was stretched to such an extent that the xylem strand had been completely straightened, with the collapsed parenchyma closely pressed against it. In fact the appearance was exactly similar to that obtained when a coiled core is pulled out by hand to its fullest extent. It is evident that the usual cell collapse and subsequent coiling had proceeded until the bulb had reached the point at which it escaped from the tube. Contraction of the root core still continued, with the result that cell collapse and subsequent contraction in the lower part exerted a pull which straightened out the previous contraction and coiling of the upper part. The petiole base had increased in thickness so that it fitted the tube closely, and it showed some twisting with crumpling and folding of its tissues as though extension in length had been forcibly prevented.

(4) In a very few seedlings weakness of or damage to the root tube had allowed escape of the lengthening petiole, which continued its growth outwards as a loop in the soil. The extended portion was sometimes curled or twisted. This abnormality was noted by Hildebrand, who found that after extrusion of the petiole no further lowering of the bulb took place. From this fact he concluded that lowering of the bulb is brought about entirely by downward thrust of the growing petiole. To confirm this he performed the experiment of slitting the cortical tube and drawing out the petiole at an early stage of development. The petiole continued lengthening as a loop in the soil and the bud remained stationary. Unfortunately Hildebrand gives no information



about the structure or behaviour of the root core in these circumstances. In the one instance examined by the present author, the petiolar loop occurred close to the bud which had also escaped from the tube. The root core was stretched taut and in the same condition as described; see (3) above.

*Comparison with other species of Oxalis in which a similar method of bulb lowering has been recorded or observed.*

*Oxalis Coppolieri* Tod. Hildebrand describes the seedling of this species as being similar to *O. rubella* Jacq., except that there is no swollen region in the root. The expanding bulb breaks laterally out of the root tube at a level undefined by any change in thickness of the root. Part of the petiole is sometimes extruded from the tube in a loop which may show twisting or contortion. This may be compared with the abnormalities of *O. rubella* described in (3) and (4) above. Hildebrand figures one instance in which the root tube was completely broken through and its edges separated owing to lengthening of the 'core' (i.e. the petiole) above the bulb, but says nothing about the condition of the 'core' (root) below the bulb.

*Adult plants of O. Coppolieri Tod., O. flava* L., *O. fabaefolia* Jacq. In all these Hildebrand describes a long slender water-storing root descending from the base of the bulb to great depths. This root may or may not form a swollen region near its base. Lateral bulbs appear as though borne directly on the root. As in *O. rubella*, the core of the root separates from its cortex which forms a hard tube. After some uncertainty regarding the interpretation of his observations, Hildebrand (1884) finally concludes:

'dass hier wirklich die scheinbar aus der Wurzel entstandenen Brutzwiebeln, welche aus der harten Rinde dieser hervorbrechen, nicht von einem inneren Wurzelstrange entspringen, sondern dass dieser vermeintliche innere Wurzelstrang die Stammachse ist. Dieselbe verlängert sich nämlich an ihrer Basis dort, wo die alten Zwiebeln festgesessen haben, oft derartig nach abwärts, dass sie das weiche Innere der Wurzel vor sich herdrückt, und wenn nun an der Stelle, wo diese Stengelachse aufhört, sowie an den etwas höher gelegenen Theilen an dieser fadigen Achse die Brutzwiebeln auftreten, und bei ihrem Wachstum die sie anfangs verdeckende harte Wurzelröhre durchbrechen. . . '

The downward-growing stem axis is said to be often contorted or coiled within the tube and sometimes part of it escapes from the tube in wormlike twists (see loc. cit., Plate I, Fig. 3 of *O. fabaefolia*). Beyond the statement that the true root core is crumpled by reason of downward pressure of the descending axis, nothing is said of its structure or behaviour.

The present author has examined young plants of *O. flava* grown from bulbs sent from S. Africa by Captain Salter in 1938. A slender shoot grows upwards from the bulb bearing scale leaves within the soil and closely set petiolate foliage leaves above it. From the base of the bulb there descends a long slender root bearing small lateral roots at intervals and becoming enlarged into a spindle-shaped swelling near its tip. Within the bulb at the base of the slender stem axis is an axillary bud which will become the next season's

bulb. The structure of the root resembles that of *O. rubella* seedling, the most prominent tissue being the large-celled secondary phloem parenchyma. Cork cambium derived from the pericycle produces a single layer of cork which splits away from the surrounding endodermis, outside which is a narrow cortex. Thus a root core similar to that of *O. rubella* lies enclosed in a cortical tube. Contraction of the core is brought about by means of the collapse of horizontal plates of cells with consequent contortion of the xylem strand. This is accompanied by downward extension of the slender stem axis carrying with it the lateral bud referred to above. At intervals above this, smaller buds may occur which later give rise to bulbils. Enlargement of the buds causes them to burst out through the tube. Extrusion of the main bulb occurred at distances of from 7 to 12 cm. below the old one. The thin cortical tube is very brittle and parts of it became completely cast off, thus exposing parts of the wrinkled contracting core and of the stem axis; in one specimen the whole of the slender threadlike stem was exposed. The lateral roots are of endogenous origin and pass out through the cortex into the surrounding soil, thus forming obstacles to the smooth downward movement of the contracting core. Their outward course is at first horizontal, but after cell collapse has begun they are directed obliquely upwards from the point of connexion of their vascular system with the xylem of the main root. This demonstrates that the central region of the contracting core offers less resistance to contraction than the outer layers. An outward kink occurs in the xylem strand at each point of connexion of a lateral root. At the stage observed the periderm of the main core was continuous with that developing in the upper part of the rootlet, the further younger part still retaining its cortex. As the main root core contracts that part of the rootlet which lies free in the dead cortex appears to be pulled downwards and inwards, slipping out of its cortex as an arm is withdrawn from a sleeve. Final stages of this process were not observed.

The small amount of material grown did not suffice for complete investigation of the later stages of contraction, so it is not known whether 'collapse contraction' is succeeded by spiral coiling of the root core as in *O. rubella*. Further supplies have not so far been available.

#### DISCUSSION

The behaviour of the seedling of *O. hirta* is a remarkable example of the co-ordination of events and processes in order to bring about the lowering of the first bulb to a suitable depth in the soil, upon which depends the future success of the individual. No complete explanation of all the processes concerned in the descent of the bulb can be drawn from the work so far carried out. The material has sufficed for little more than observation of the seedling structure and behaviour. Little information has been to hand about the precise conditions under which *O. hirta* lives in its native habitat in S. Africa. It is noteworthy that in Britain germination and seedling growth take place immediately the seed is shed, in November or early December, towards the

end of the growing season of the adult, the result being a resting shoot or bulb; the mature bulb sprouts at the beginning of the growing season in August when it produces active, aerial shoots. Development and ripening of bulbs in the adult is taking place while active lengthening of aerial shoots is declining or at a standstill, from December to April when the length of day is increasing. Development of the seedling extends over the period December to April and corresponds to the same phase of the life history, viz. non-elongation of the aerial shoot while storage and bulb development proceeds. There was no appreciable difference in appearance or structure between seedlings grown here and those received from S. Africa, despite differences of seasons and conditions.

The main question concerns the lowering of the bulb in the root tube. Is its descent brought about by pull of the contracting root or by downward thrust of the elongating petiole? Hildebrand considered that the bud is forced downwards by the growth of the petiole within the tube. He says: 'es fängt der Blattstiel an seiner Basis an sich zu strecken und führt dabei die Endknospe nach abwärts, den inneren Gewebestrang der Wurzel vor sich herschiebend', and again: 'dass der Stiel des einzig bleibenden Blattes des Keimlings in dem an der Basis der Cotyledonarscheide gelegenen Theile sich derartig streckt, dass er das Innere der Wurzel nach abwärts vor sich her treibt, bis er schliesslich in dem spindeligen Wasserspeicher angelangt ist.' He recognizes the root as a 'water storer', especially the swollen part of it. He pays little attention to the behaviour of the contractile parenchyma beyond assuming that water is withdrawn from its cells which consequently shrink or collapse, thus bringing about the separation of the core from the endodermis. He has apparently not seen the alternating bands of empty and turgid cells and says nothing about protoplasmic contents. He affirms that the sinking of the bud is initiated by the downward extension of the petiole. This *compresses* the 'root core' as it separates from the tube; meanwhile the 'root core' offers *resistance to compression* which causes slight twisting of the elongating petiole. The root and petiole are visualized as acting in opposition to one another, with the petiole always the stronger. It is evident from the present work that far from merely offering *passive resistance* to compression from above, the root core is *actively contracting* and exerting a downward pull at least in so far as cell-collapse contraction is concerned. This is most clearly shown in the abnormal seedlings described above. Collapse of cell layers is proceeding in an orderly sequence from above downwards in a length of root core which has become fixed at its upper end (through extrusion of the bulb from the tube) and is therefore unable to continue shortening. Collapse of cells in the basal region stretches out the effects of the previous contraction and coiling in the upper parts, pulling the core taut.

Collapse of cells taking place in secondary phloem parenchyma is a process common to all the forms of contractile roots in the genus *Oxalis* so far examined. The contracting organ or 'core' always consists of the stelar tissues and a part of the pericycle covered by a thin periderm. The nature and behaviour of

the tissues are similar in all respects in slender roots, such as *O. flava* and *O. rubella* seedlings, and in the much larger and thicker roots of *O. incarnata*, *O. cernua*, *O. lasiandra*, &c. In the larger roots the 'core' is no longer enclosed within the cortex and is visible and free in the soil. In *O. incarnata*, *O. cernua*, and others, root contraction is accompanied by correlated downward extension of a slender underground stem (bulb axis) fixed above by adventitious roots. In a number of 'stemless' *Oxalis* species, including *O. lasiandra*, *O. Martiana*, and *O. brasiliensis*, no part of the plant above the bulb is fixed, and as contraction proceeds the bulb, with any leafy parts remaining, is drawn down into the soil (Fig. 9). Here it is obvious that no force other than the pull exerted by the contracting root can account for the lowering of bulbs to depths of several inches in the soil.

The *spiral coiling of the root core* on itself in *O. hirta* is more difficult to explain. It is essential for lowering of the bulb as far as the swelling. Collapse contraction alone cannot effect this because it takes place in a limited length of core terminated by the swelling, beyond which in the narrower part of the root collapse is not continued. Downward growth of the petiole is not necessarily so limited. Coiling follows on regularly after cell collapse has begun; when the only remaining continuous tissue through which a force could be transmitted is the central xylem strand with its surrounding cambium. This has already suffered contortion or spiralling brought about by collapse of the surrounding parenchyma. It is possible that pressure due to continued growth of the descending petiole may now make itself effective in producing the coiling. Pressure on an already existing enclosed continuous spiral will cause it to assume a second spiral. An experiment, using a spirally coiled wire enclosed in a glass tube, showed that pressure applied to its end produced spiralling of the coil. Hildebrand cites some instances in which the resistance of the root to compression becomes effective, forcing the core upwards in a loop beside the petiole base so that transverse sections pass through the root core twice and the petiole once. For this to happen the bulb and petiole must be appreciably smaller than the 'bore' of the tube, thus allowing space for the upward stretching of the core. Such an occurrence has been seen by the present author only in the last stages of lowering, when the bulb is settling into the swollen region; here there is evidence of relaxation and slight upward extension of the coil (see above p. 246).

It may be significant that at this stage growth of the petiole has practically ceased. With regard to this final stage in which relaxation occurs, displacement of the coil shows that it is no longer effective as a lowering agent, neither is the contracting part beneath it effectively connected with the bulb. Whatever the effective factor, the lowering of the base of the bulb will necessitate



FIG. 9. *Oxalis lasiandra*. Mature bulb drawn down in soil by collapse-contraction of fleshy root. ( $\frac{1}{4}$  nat. size.)

lowering the attached end of the root core. Since, owing to the widening of the tube near the swelling, the core is no longer closely confined laterally, it can avoid compression by means of lateral displacement forming an upward-pointing loop with a kink at the bend. The descending attachment will exert a pull on the upper part of the coil which will straighten out the turns of this inverted part of the loop. Further expansion of the bulb would cramp and fix the looped part. While it is true that spiral coiling might be the result of compression, as in the case of the spring previously referred to, it is also possible that coiling might be spontaneous, as in tendrils (where inversion occurs) and in the fruiting stalks of *Cyclamen* and *Vallisneria*.

The abnormalities do not seem to throw any clear light on the normal process. In those instances where descent of the bulb is checked owing to its escape from the tube (case (3) above), the straightening of the coil is evidence of the effectiveness of cell collapse-contraction. It also shows that if coiling plays an active part it exerts less force than the collapse-contraction. In such cases the compression or contortion of the basal part of the petiole demonstrates its power to push. In so far as that part of the petiole which has finished elongating is firmly held by the walls of the tube and the intercalary growing region is short, the growth pressure would be applied efficiently, as in the growth of a normal root. In this connexion, however, it must be noted that whereas in the author's *O. hirta* the petiole practically fills the tube and approximates to a cylindrical form, in Hildebrand's *O. rubella* the petiole is more slender, flattened or crescent-shaped in section, and judging from his figures it lies loosely in the tube (Fig. 4). That elongation of the petiole may extend outwards as a loop through a slit in the tube above the fixed bulb gives little evidence of the magnitude of growth pressure. Hildebrand's contention that this elongation is a *proof* that the bulb is pushed down by the petiole fails in the absence of evidence that the bulb was still free to move.

It is clear that the explanation of the lowering of the bulb must lie between the following two extremes, but exactly where it lies the evidence does not enable us to decide: (a) The bulb is pulled down by the growth and collapse-contraction of the root core followed by *active* coiling of the core; the elongating petiole allows the bulb to descend, the coiled core acting as a spring buffer in case elongation does not exactly keep pace with the petiole. Coiling ceases to be effective when the bulb has reached the root swelling, the 'collapse'-contraction of the core in this part serving to empty it and make way for the bulb. The growth of the bulb and petiole are then correlated, the root core is displaced, and its coils distorted. (b) The petiole pushes the bulb down the tube, contraction of the core serving to clear the path below it. Coiling which is *passive* would serve the same purpose and perhaps prevent irregular crumpling of the contracted core under pressure from the petiole which might otherwise block the tube.

Judging from the abnormalities showing a stretched core, and by analogy with the water-storing roots of such species as *O. incarnata*, it is clear that growth and collapse-contraction exert a downward pull. The outstanding

difficulty lies in assessing the part played by the petiole and by the coiling of the core.

Critical experimental evidence would be very difficult to obtain owing to the small size of the structures concerned and the consequent difficulty of manipulation. Also growth processes are involved which adjust themselves to the interplay of forces with the margin of uncompensated strain at a minimum. It is unlikely, therefore, that the coil would expand or contract, as the case might be, more than very slightly, and the amount might well be less than the errors of manipulation.

#### SUMMARY

After the expansion of one petiolate foliage leaf, the plumular bud of *Oxalis hirta* L. develops into a resting bulb which is lowered from the soil surface to a depth of from 3 to 10 cm. within a tube constituted by the dead cortex of the primary root.

Lowering of the bud is accompanied by (a) contraction of the root core, separated from the endodermis-lined cortical tube and free to move downwards within it; and by (b) correlated intercalary extension downwards of a section of the foliage leaf-petiole inside the tube, its upper end being fixed by the close grip of the cotyledonary tube.

Considerable shortening of the root core is brought about by (a) collapse of horizontal plates of the secondary phloem parenchyma after loss of their protoplasm and watery sap, followed by (b) spiral coiling of the core upon itself.

Comparison with root contraction in other species of *Oxalis* together with evidence from abnormal seedlings shows that root contraction by means of 'cell collapse' exerts a downward pull, and is probably responsible for at least the initial stages of the lowering of the bud. Coiling of the core associated with downward extension of the petiole brings the bulb to its final position.

There is some evidence that the root core offers resistance to spiral coiling in its final stages and that this resistance may become effective when the petiole has ceased to elongate and is no longer closely confined by the widening tube near its base.

It has not been possible to determine whether coiling of the core is active or passive, nor to assess the part played by the petiole in lowering the bulb.

In conclusion, I wish to thank Professor Thoday for his stimulating and helpful criticism, especially in regard to interpretation of the facts. I am very grateful to Dr. M. R. Levyns of the University of Cape Town, and Paymaster Captain Salter, R.N., of Cape Town for material and information from S. Africa. My thanks for material are also due to the Curators of Kew Gardens, and of the Royal Botanic Garden, Edinburgh, and to Mr. Exell of the British Museum for identifications.

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# Studies in the Development of the Inflorescence

## I. The Capitulum of *Bellis perennis* L.

BY

W. R. PHILIPSON

With Plate VII and four Figures in the Text

SINCE the work of Grégoire (1938) reawakened interest in the meristems of the flowering apex, some work has been done by morphologists on the histogenesis of the inflorescence (Brooks, 1940; McCoy, 1940; Satina and Blakeslee, 1941; Reeve, 1943; McLean Thompson, 1944 [1946]). The development of the inflorescence had been studied, previously, largely with reference to its external morphology. It is true that economic botanists have built up a valuable literature on 'blossom-bud differentiation', but, in general, they have confined their descriptions to features which serve the practical ends they have in view. Occasional references to the ontogeny of the inflorescence have also appeared as part of more general morphological papers. The following papers are chosen for citation because of the useful illustrations of young inflorescences they contain: Jones and Emsweller (1911), onion; Barnard (1932), Barnard and Thomas (1933), Snyder (1933), and Winkler and Shemsettin (1937), grape; Barnard and Read (1932-3), orchard fruits; Thompson (1933), cabbage; Abbot (1935), citrus fruits; Gore (1935), cotton; Aaron (1936), apple; Tsu Kiang Yen (1936), *Ribes*; Reece (1942), avocado. Physiologists interested in photoperiodism have also investigated the morphological changes which accompany the onset of flowering (Biddulph, 1935; Bothwick and Parker, 1938; Hamner and Bonner, 1938). Nevertheless, in 1941 Forster found it necessary to state that 'our knowledge of the comparative histology of vegetative and floral apices is at present meagre', and three years later Rickett (1944) wrote: 'There was little detailed anatomical work on inflorescences in the 19th century; Hieronymus made a beginning in 1872 and 1886, Goebel in 1884, and Schumann in 1889, but this field remains relatively untilld until the present day'; and 'In trying to put the study of inflorescence upon a modern basis, we encounter the same lack of morphological study which exercised Schleiden a hundred years ago'.

It is proposed, in a later paper, to review the literature relating to the development of the inflorescence, but in the first place it is intended to describe, in the present series of papers, the morphological changes which occur at the stem apex during the period between the production of foliage leaves and flowering. For the present these studies will be limited to types chosen from the Compositae, Dipsacaceae, and related families, with a view to a comparative study of the ontogeny of the capitulum.



*BELLIS PERENNIS* L.

## I. THE TRANSITION FROM THE VEGETATIVE TO THE REPRODUCTIVE STATE

The capitula stand singly at the ends of leafy shoots. Buds do not form in the axils of the two leaves immediately below the capitulum; all other leaves subtend buds, but normally only the highest of these develop into leafy shoots which continue the axis sympodially. These lateral axes in turn soon develop terminal capitula, and although two or more capitula may thus be formed close together, a loose cluster of this kind cannot be regarded as a compound inflorescence because the constituent heads are separated by leaf-bearing axes. In other words each apex, after passing from the vegetative phase, develops into one capitulum only.

The transition from the vegetative phase to the reproductive is not gradual; the primordium of the uppermost leaf is formed no differently from those of the preceding leaves. But before this primordium has developed beyond the very youngest stages, the apex of the axis has become transformed. To appreciate the morphological changes which lead to the development of the inflorescence, it is necessary to become familiar with the appearance of the vegetative apex.

The vegetative apex of *Bellis perennis* presents no unusual features. The apex during the phase of maximal area is broad and flat (Pl. VII, Fig. 1), and even after the appearance of a young leaf primordium, that is at the time of minimal area (Schmidt, 1924) when its curvature is greatest, the arch is very low. The young primordium immediately grows faster than the apex, and since the primordium and the apex are originally of approximately the same height it follows that the leaf primordium is never over-topped by the stem apex so long as the latter remains in the vegetative condition. As soon as the leaf primordium is formed its cells begin to vacuolate, except for the apical and marginal meristems and for the median plate of provascular meristem.

The cells at the stem apex are arranged in a pattern typical of many dicotyledons. The tunica is two layers deep and regular; within it the arch of the apex is filled with a lens-shaped mass of meristematic tissue formed of layers of cells more or less parallel with the tunica, but not entirely regular. As in the tunica, most of the cell-divisions in this meristematic mass are radial with reference to the arch of the apex. Divisions approximately parallel with the surface also occur, and by them the number of cell-layers is increased from usually 5 to 7 layers at the centre to 9 or more at the flank of the meristematic mass. Each successive layer is shorter than those outside it, and in some sections the series appears to end in a single central cell. Some differentiation can be discerned even within this lens-shaped mass of meristem (Pl. VII, Fig. 2): the central zone is composed of comparatively large cells which divide rarely; it is surrounded by a zone of smaller, more deeply staining, and more actively dividing cells—the flank meristem of Majumdar (1942) (Pl. VII, Fig. 2, below the black arrows). From the lower side of the central part of the apical

meristem, cells are divided off almost as regularly as from a cambium and develop into a file meristem (Pl. VII, Fig. 2, above the white arrows). This structure is not present in all vegetative apices of *Bellis*; it is probably a transient state, perhaps confined to the earlier part of each plastochrone. While the file meristem of many angiosperms originates from the apical meristem in a regular manner, the only description of a cambium-like zone of cells in this position which I have been able to find is given by Ball (1941) in his paper on *Phoenix*. As might be expected, the structure of the palm apex is very different in other respects from that of *Bellis*; the significance of the presence of a 'cambium'-zone between the promeristem and the file meristem cannot be assessed until the disposition of the apical cells has been studied in a wide range of plant affinities at all stages of their life-histories. Nearer the surface of the stem apex, the cells lying below the meristematic mass are not so active and pass into the maturing tissue of the stem without the intervention of a rapidly dividing zone.

Turning now to the youngest stage in the development of the inflorescence (Pl. VII, Fig. 3), the onset of flowering can be recognized immediately by the more strongly arched apex which now overtops the primordium of the highest leaf. At a later stage this leaf will outstrip the apex again, but by that time the form and structure of the apex cannot be confused with that of the vegetative apex. There is some superficial resemblance between a longitudinal section through a very young inflorescence and one cut through a vegetative apex at right angles to the median axis of the youngest leaf-primordium; but an examination of the whole series of sections reveals in the latter the presence of a leaf primordium at the same level as the apex.

A comparison of the sections shown in Pl. VII, Figs. 1 and 3, in each of which the youngest leaf-primordium is cut medianly and at approximately the same stage of development, shows that, in the latter, the leaf has been thrust to one side by the swelling rudiment of the inflorescence. The increase in height of the apex in Pl. VII, Fig. 3, has not been caused by any marked increase in the number of cells but rather by an increase in depth of the individual cells at an earlier stage than in the vegetative apex. A comparison of the two sections shows that there are approximately the same number of cells in each, and that the arrangement of the cells in Pl. VII, Fig. 3, could be produced from that in Pl. VII, Fig. 1, by distortion into an arch of greater curvature. The increase in depth of the cells both of the meristematic mass and of the tissue immediately below it appears, literally, to have distended the apex. The morphological appearance of the inflorescence primordium therefore suggests that the primary change which leads to the onset of flowering is an elongation of the cells immediately below the apical meristem. This might be due to (i) an increase in their osmotic pressure, or (ii) an increase in the plasticity of their cell-walls, or (iii) a decrease in the permeability of their membranes. The fact that the increase in size is principally along one axis of the cells suggests that the factor involved is the plasticity of the walls. Went (1935) records that auxin has an effect on the plasticity of cell-walls.

## II. THE DEVELOPMENT OF THE CAPITULUM

(a) *The formation of bract primordia.*

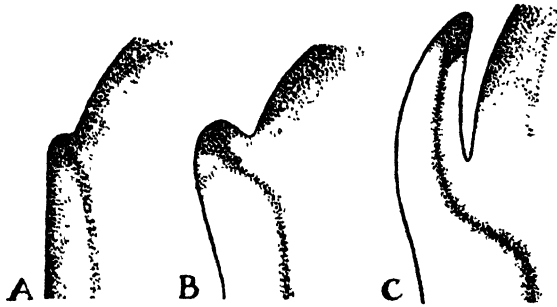
The inflorescence primordium, once formed, grows in height by the active division of its apical meristem. This meristem retains its domed shape, and not until it has attained the height of  $150\mu$  do the first primordia of the lateral organs (bracts) appear. The sudden interruption in the production of lateral appendages coincides with the arching of the apex to form the inflorescence primordium, and is no doubt due to the same cause which, it has been suggested, may be an increase in the pressure exerted by the internal tissue. Owing to the absence of appendages the tissues are laid down with something of the regularity found in the root. The apical meristem has the same lenticular shape and zonation as in the vegetative apex and the cylindrical axis below it is formed as in the leafy shoot, except that the outer layers remain meristematic much farther down the apex, forming an extensive peripheral meristem from which the involucre bracts and florets will arise (Pl. VII, Fig. 4, white arrows). The section shown in Pl. VII, Fig. 4, passes through the centre of an inflorescence which was just about to lose its regularity of growth. The complete series of sections through this inflorescence primordium shows that, on each side of the plane of the section figured, primordia of the first two bracts had already appeared, but the tissues in the plane of the section had not yet been affected.

The initiation of the bracts is unlike that of the foliage leaves. The latter arise as vertically growing primordia to one side of the apex, whereas the bract primordia arise well below the apex of the inflorescence rudiment. Moreover, the bract primordia form as very localized cell-divisions, in contrast with the leaf primordia which are already broadly inserted on the stem apex at their first appearance. As the inflorescence primordium enlarges the involucre bracts appear successively in a compact zone to form a double circle midway up the inflorescence rudiment, dividing the peduncle from the receptacle. There is every reason to suppose that the thirteen bracts which very commonly make up the involucre in *Bellis perennis* are initiated in their theoretical numerical order. It would be necessary to cut serial sections of a very great number of capitula to obtain all the stages necessary to prove this, but the sections which have been made of young capitula with varying numbers of bracts developed show invariably the bract primordia in the expected theoretical positions, no gaps being left in the series to be filled later. No attempt has been made to ascertain whether the florets are laid down in the theoretical order, but it is interesting that none is initiated until the last bract has appeared, and they do, of course, arise in a general acropetal manner.

The cell-divisions which lead to the formation of the individual bracts first appear in the second tunica layer. The primordium so formed grows by apical and marginal meristems as in the case of the foliage leaves.

(b) *The vascular system of the peduncle and involucre.*

The initiation of the bracts, and later of the florets, so far below the apex in comparison with the foliage-leaf primordia, results in the provascular tissue of the inflorescence having a somewhat different origin from that of the leaf-traces. In the vegetative stem growth is predominantly in length, and we have seen how the meristematic zones are confined to the apex, where leaf primordia are formed and the main tissue-zones of the axis are defined. In the capitulum, however, the direction of growth is as much lateral as vertical, and, as McLean Thompson (1944) has pointed out, it is in these circumstances that a dome-shaped meristem such as Grégoire (1938) called the *manchon meristematique* is formed. The inflorescence rudiment,



TEXT-FIG. 1. Three stages in the development of an involucre bract (compare with Pl. VII, Figs. 9, 10, and 11). Meristematic tissue stippled. A. The vacuolation of the middle layers of the peripheral meristem is defining the provascular meristem up to the level of the young bract-primordium. B. Vacuolation has continued above the level of the bract, but the cells of the bract trace remain meristematic. C. The peripheral meristem above the bract is now dividing to form a provascular meristem.

therefore, is covered by an hemispherical meristem and the process of cell-maturation is at first confined to the central tissue (Pl. VII, Fig. 4). This peripheral meristem is concerned not only with the production of the involucre bracts and the florets, but, as will be described later, with the growth in breadth of the central mass of the capitulum; in addition it is from this meristem that the provascular tissue of the inflorescence is derived. In some respects this delayed maturation of the cortical zone recalls the condition described by Louis (1935) in the leafy shoots of *Taxus*, by Esau (1942) in *Linum*, and which Esau (1943) suggests is typical of gymnosperms and small-leaved angiosperms, that is, plants in which the size of the lateral appendages is small in comparison with the diameter of the axis.

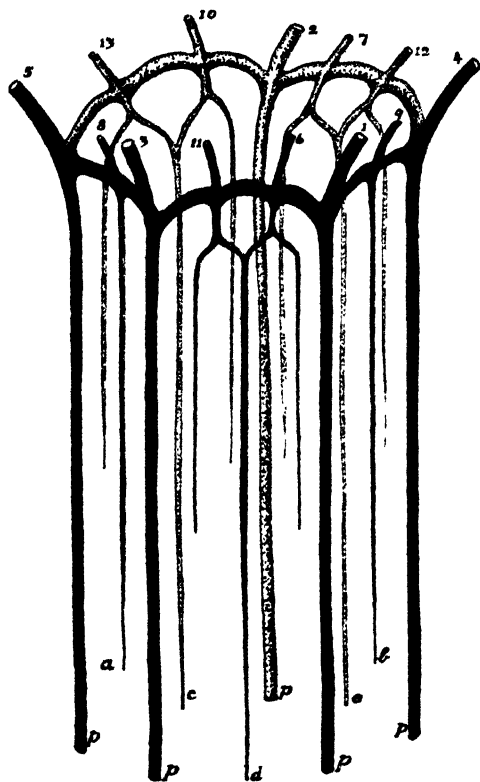
Reference to Pl. VII, Figs. 9, 10, and 11, and Text-fig. 1, will show the origin of the provascular tissue from the peripheral meristem. At the time when the first cell-divisions in the production of an involucre bract are taking place in the second tunica layer and the outer corpus, the peripheral meristem below the level of the bract is seen to be affected by cell-maturation in its central layers (Pl. VII, Fig. 9, Text-fig. 1A), so that an inner provascular meristem (indicated by arrows on the photograph) is separated from an outer

peripheral meristem. The provascular meristem, therefore, is cleft from the peripheral meristem by the upward progress of vacuolation in the cortex. Grégoire (1938) describes a precisely similar mode of origin for the provascular tissue in the torus of *Ranunculus sceleratus* (see his Fig. 226).

The vacuolation of cortical tissue takes place continuously round the base of the inflorescence rudiment, so that a cylinder of provascular meristem is divided from the peripheral meristem. This cylinder is continuous below with the vascular system of the leafy stem and above with the, as yet, undivided peripheral meristem of the upper part of the inflorescence rudiment. This provascular cylinder can be seen in longitudinal section in Pl. VII, Fig. 4 (black arrows), and at a later stage in Pl. VII, Fig. 6. The cells of the peripheral meristem become increasingly vacuolated towards the centre of the receptacle. In view of its origin from the inner cells of the peripheral meristem, the provascular meristem is not highly meristematic at its inception, but the cells in the position of the future traces of the outer involucre bracts soon regain their deeply staining quality. Pl. VII, Fig. 10, and Text-fig. 1B show that the vacuolation of cortical cells does not affect the meristematic cells immediately within the bract primordia (indicated by arrow), which, therefore, remains connected by meristematic cells to the provascular cylinder. It is from these cells that the bract-trace is derived (Pl. VII, Fig. 11, and Text-fig. 1C). Pl. VII, Fig. 10, also shows (at X) the formation of the 'leaf-gap' by the maturation of the provascular meristem immediately above the bract.

In the region of the peduncle the provascular meristematic cylinder becomes divided, by the maturation of longitudinal bands of its cells (the primary medullary rays), into a number of provascular strands which become increasingly 'meristematic' in character. Five of these strands are considerably larger than the remainder, and extend without diversion or lateral junctions from their insertion among the leaf-traces of the uppermost leaves to the top of the peduncle, where (as already described) they retain connexion with the provascular meristem of the involucre bract-primordia and form their mid-ribs. At the level of the involucre the provascular cylinder remains unbroken and from it vascular tissue develops, running transversely, to form a continuous 'collar' of vascular tissue around the base of the capitulum (Text-fig. 2). The mid-ribs of the remaining eight involucre bracts, like the outer five, form concurrently with their bracts and retain connexion with the provascular cylinder. But the course of these eight bract-traces below the 'collar' is different from that of the five principal peduncle strands. Their course can be understood most readily by referring to Text-fig. 2. Two of the strands, (a) and (b), stand singly between the principal bundles; these two bundles run undivided throughout the peduncle, but in all capitula examined they end blindly without any connexion with the vegetative vascular tissue. In more mature capitula, no doubt, such a connexion is made, but in that event the vascular tissue would be derived by the secondary modification of non-meristematic cells. The remaining six bract-traces are distributed in pairs between the principal bundles; both bundles of each pair divide immediately below

the 'collar', and of the four bundles so formed the central two unite to form a single strand. The two lateral bundles run for some distance down the peduncle but sooner or later disappear. With their disappearance the peduncle acquires a rather symmetrical appearance, with five principal strands alternating with five lesser strands. The three strands, (c), (d), and (e),

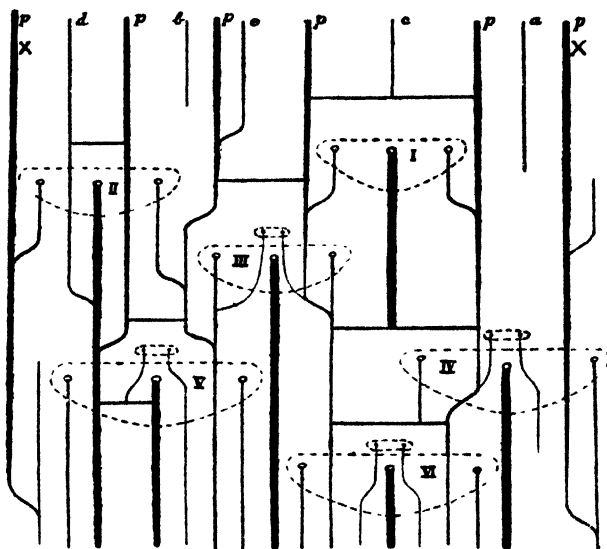


TEXT-FIG. 2. The vascular system of the peduncle and the involucre. The traces of the thirteen bracts are numbered in the order in which they are laid down; they are connected by a continuous ring, the 'collar'. *a, b*, minor strands which do not reach the vegetative system; *c, d, e*, minor strands which reach the vegetative system; *p, p*, five principal strands of the peduncle.

now under consideration, however, are stronger than strands (*a*) and (*b*), and unlike them persist until they make connexion with the leaf-traces.

The vascular system described above and figured is generalized to some slight extent; it is subject to some variation between individuals. In particular, since one plant can be examined only at one stage in its development it is difficult to determine precisely what portions of the strands are derived from primary meristematic tissue and what from cells which have reverted to the meristematic condition. It is considered that the strands so far described are derived, at least in strong plants, from the original provascular cylinder, though this cylinder persists only in the most tenuous form in the positions where the minor bundles eventually develop. The fact that these bundles

taper towards their lower extremities suggests that they are extending downwards into the already partly mature parenchyma. Moreover, in the mature capitulum each involucre bract is furnished with a pair of lateral traces in addition to the mid-rib already considered; these lateral traces appear at a comparatively late stage in the development of the capitulum, and are derived from cells which had previously been in a parenchymatous state. The lateral bract-traces are inserted on the 'collar', but do not continue below it into the peduncle.



TEXT-FIG. 3. The junction between the traces of the upper leaves and the strands of the peduncle; diagram of strands opened and drawn in one plane, the strand marked X and its branches depicted twice. Lettering as in Text-fig. 2. Leaf-bases and their axillary buds indicated in discontinuous outline, and numbered from above downwards I-VI.

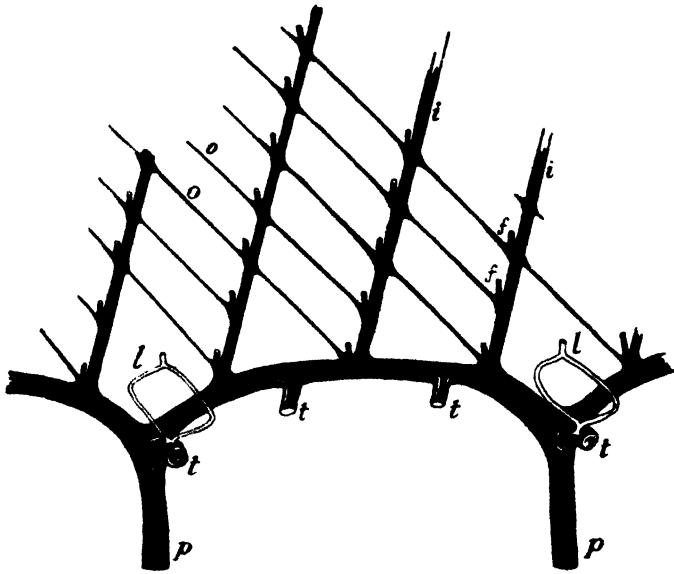
Text-fig. 3 shows, for one individual plant at the stage of development represented in Text-fig. 2, the manner in which the vascular strands of the peduncle link up with those of the leaf-traces of the upper leaves.

### (c) *The receptacle and the florets.*

The development of the essential features of the peduncle and involucre has now been described. In order to trace the development of the receptacle and the florets it is necessary to return to the stage when the last bract-primordium had formed and the first floral primordia were appearing (Pl. VII, Fig. 5).

As has already been stated, the arrangement of cells at the extreme apex of the young inflorescence-rudiment does not differ materially from that of the vegetative apex. The manner of cell-formation from a lens-shaped meristem is essentially the same, but the delay in the production of lateral appendages and the increase in breadth leads to modification of the further growth.

As the hemispherical receptacle enlarges, the cells of the central tissue increase in size and become vacuolated, and large intercellular spaces appear. As we have seen, however, the peripheral layers remain actively meristematic, dividing both at right angles to, and parallel with, the surface. A comparison of sections through successively older capitula shows that the number of vertical rows of vacuolating cells in the central tissue of the receptacle increases with age. This is brought about partly by some longitudinal divisions in the file meristem, but mainly by the vacuolation of the inner cells of the



TEXT-FIG. 4. Portion of vascular system of receptacle. *f, f*, flower-traces; *i, i*, strands following the short parastichies; *l, l*, flower-traces inserted directly on to bract-traces; *o, o*, strands following the long parastichies; *p, p*, principal strands of the peduncle; *t, t*, involucre bract-traces.

peripheral meristem. This meristem, therefore, although continually dividing, scarcely increases in thickness, but rather moves slowly outwards upon the increasing central tissues, to whose growth it contributes. A similar manner of growth has been described by Grégoire (1938) in the torus of various flowers. In spite of the steady vacuolation of its inner cells the peripheral meristem increases in depth; on the other hand, the lens-shaped apical meristem becomes less pronounced as growth in length falls off, until the central zone of the corpus disappears and the receptacle is clothed in a uniform meristematic mantle.

The vascular system of the receptacle forms a peripheral network below the flowers, to each of which a single strand is given off (Text-fig. 4). The system develops upwards from the ring of vascular tissue at the base of the involucre by the process of splitting off from the peripheral meristem which has already been described. The earlier-formed flower-primordia appear well in advance of their provascular strands, but the splitting off of the pro-



vascular strands follows closely on the formation of the upper flower-primordia (Pl. VII, Fig. 12). The increase in breadth of the provascular strands is clearly illustrated in Pl. VII, Fig. 13, which represents the vascular supply of a flower rudiment. The strand can be seen to be composed of tiers of cells, and evidently the cells in each tier are genetically related.

In view of the fact that the position of the provascular strands in the receptacle is governed by that of the flowers, it is perhaps not surprising that the vascular bundles run along the parastichies on which the flower primordia lie. Nevertheless the resultant network is a very beautiful structure (Text-fig. 4). In the capitulum of the daisy the flowers lie on 21 long and 13 short parastichies, and the fine vascular bundles which grow up from the 'collar' which encircles the top of the peduncle gradually build up a network of 13 veins crossed by 21 other strands. The 13 veins being nearer the vertical become more strongly developed, but each is connected to its neighbours by the fainter 21 veins, as though by the rungs of a ladder.

The regularity of this network, with lozenge-shaped gaps, tends to be disturbed at its base, where it is inserted on the 'collar'. Here the outermost 'whorl' of flowers, which are carried outwards on the base of the involucre (see Pl. VII, Fig. 8), have their vascular supply inserted directly on the mid-ribs of the bracts, not on the 'collar'. The vascular supply of these flowers ends blindly and does not, as with the upper flowers, give off branches to its neighbours. Text-fig. 4 indicates the relationships between the bundles in a small part of the base of the receptacle.

The development of the flower rudiment is outside the scope of the present series. It may be stated, however, that the primordia are first formed by divisions in the second tunica and first corpus layers. The hemispherical primordium immediately flattens until it is stud-shaped, when its circumference grows up to form a cup (Pl. VII, Fig. 7). By this time the tissue of its base has become vacuolated, leaving a single provascular strand. This strand later branches into dorsal and ventral 'carpellary' strands (Pl. VII, Fig. 13). It is interesting to note, in connexion with the 'order of flowering', to which so much importance has been attached by some writers on the inflorescence, that although the flower primordia are laid down acropetally, the outermost flowers (comprising about two 'whorls') are soon outstripped in stature by the younger primordia. It is these backward flowers that form the ray-florets, and their stunted state is partly due to the lack of stamen rudiments. The appearance of the developing capitulum suggests that these flowers lack the room needed for full development. Their period of anthesis is simultaneous with, or may even precede, that of the outer disc-florets; the order of anthesis, therefore, does not depart from that of initiation.

### III. DISCUSSION

In emphasizing the differences between vegetative and reproductive stem-apices Grégoire (1938) attempted to show, on evidence which he himself admitted to be insufficient, that the rudiment of the inflorescence arises

as a lateral emergence on the flank of the vegetative apex. Descriptions published since that time, such as those cited in the introductory paragraph, do not support this view, and in some cases clearly show that the vegetative apex itself is transformed into the reproductive apex. The present work confirms that view.

Grégoire showed that the apical meristems of inflorescence primordia are fundamentally the same as those of single flowers. This reproductive type of apex he regarded as radically distinct from that of the vegetative shoot. McCoy (1940) did not accept Grégoire's antithesis between vegetative and floral apices and considered that the histogenesis of floral organs in *Frasera* was essentially similar to that of the foliage leaves. Satina and Blakeslee (1941), however, using colchicine-induced periclinal chimaeras, considered that the stamens and carpels of *Datura* were axial rather than foliar structures. In the present study of *Bellis* the transformation of the apex from the vegetative phase to the reproductive phase is described. The vegetative promeristem shows the zonation typical of angiosperms, that is, a tunica enclosing a corpus in which a central initiation zone is surrounded by a peripheral meristem and surmounts a file meristem (Kaplan, 1937; Majumdar, 1942). The same zonation is present in the young inflorescence of *Bellis*. The peripheral meristem of the inflorescence apex can be seen to be directly derived from the peripheral meristem of the vegetative apex by its extension down the flanks of the apex. As the vertical growth of the inflorescence ceases the central zone of the corpus disappears, being replaced by the peripheral meristem, which then extends continuously over the apex, becoming Grégoire's *manchon*. The *manchon*, therefore, is derived from the peripheral meristem, from which it inherits the function of initiating lateral appendages and forming the cortex and provascular meristem. The zonation of the vegetative and reproductive apices are therefore unlike, in that the central zone is lacking from the corpus in the latter; but this difference appears to be a result of different directions of growth rather than of such a fundamental nature as Grégoire attributed to it. This conclusion is in accordance with that of McLean Thompson (1944), who regards the *manchon* as an expression of growth in breadth at the apex as well as growth in length. This view of the change in the disposition of meristematic tissue seems applicable to reproductive apices, but not to those of plants in which a peripheral meristem extends well down the flank of the vegetative apex (Esau, 1942). The promeristem of such plants is more or less hemispherical, but is not comparable with the *manchon* of a reproductive apex because the corpus retains a central zone and growth is predominantly in length. Esau (1943) suggests that apices of this type are characteristic of plants in which the lateral appendages are small in comparison with the diameter of the axis.

Murneek and Gomez (1936) describe the histological changes which precede flowering in the soy bean. While the change from the vegetative to the reproductive state progresses gradually in that plant, in contrast to the sudden metamorphosis seen in *Bellis*, it is interesting that those authors describe an

increase in size of the cells of the apical meristem corresponding to that now observed in *Bellis*. Their description of the earlier maturation of the apical cells in the reproductive apex is in accord with the view of McLean Thompson (1944), that the onset of flowering is caused by a shift in the balance between meristematic growth and cell-maturation. In describing the various forms exhibited by the apex of *Cycas revoluta*, Forster (1940) regards the increased height of the apex as due to an increase in the development of the rib-meristem.

Priestley (1928) considers that the lateral appendages of the shoot arise as folds caused by the growth of all the layers of the stem apex at equal rates in all directions. He points out that in the root growth is predominantly in length and folds do not occur. It is suggested that the increase in length which has been observed in the apical cells of *Bellis* at the inception of the inflorescence primordium may be the factor which, by preventing the appearance of folds, interrupts the formation of lateral appendages between the uppermost foliage-leaf and the outermost involucre-bract.

The mode of formation of the vascular strands in the capitulum of *Bellis*, and also in the anatomically very similar torus of *Ranunculus*, may be a further expression of the growth in breadth which results from the formation of a meristematic *manchon*. Wardlaw (1944) has suggested that a characteristic of actively growing meristematic tissue is that it leaves vascular tissue in its train. In the case of a dome-shaped meristem growing outwards in all directions, the splitting off of a continuous provascular meristem parallel to the actively growing peripheral meristem would be consistent with Wardlaw's hypothesis.

The provascular strands of the peduncle and capitulum of *Bellis* appear uninterruptedly in an acropetal manner. Grégoire (1938) considered that the acropetal development of vascular strands was characteristic of reproductive apices. Modern work (Priestley and Scott, 1936; Majumdar, 1940; Boke, 1940; Reeve, 1942; Esau, 1943; Douglas, 1944) indicates, however, that this mode of development is found also in vegetative axes.

#### SUMMARY

The morphological changes which appear at the stem apex of *Bellis perennis* during the initiation of the inflorescence primordium are described. The zonation characteristic of the vegetative promeristem is modified in the young inflorescence primordium by the downward extension of the peripheral meristem. At a later stage the central zone of the corpus is replaced by the peripheral meristem which thereupon forms a continuous *manchon meristématique*.

The development of the involucre bracts and their vascular supply is described. The provascular meristem is laid down acropetally and is formed from the peripheral meristem by the maturation of the cortex.

The type of growth whereby the receptacle increases in volume is described. The initiation of the flower primordia and of their vascular supply is described up to the stage when the floral organs first appear.

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<sup>1</sup> A fuller typescript version of this paper is preserved in the archives of the Linnean Society of London, with the title 'A Study in Modern Angiospermy'.

## DESCRIPTION OF PLATE VII

Illustrating Mr. W. R. Philipson's article 'Studies in the Development of the Inflorescence.

### I. The Capitulum of *Bellis perennis* L.'

FIG. 1. Longitudinal section through vegetative stem-apex, cutting the youngest leaf-primordium medianly. The dense meristem at the apex is flanked on the left by the young leaf-primordium, which already is higher than the apex. A pronounced vascular strand extends to the tip of the leaf primordium. (× 260.)

FIG. 2. Apex shown in Pl. VII, Fig. 1, but more highly magnified. The cells at, and below, the extreme apex are comparatively large. On either flank of this central zone (below the black arrows) are smaller, more deeply staining cells. On the lower margin of the apical meristem is a zone of flat 'cambium-like' cells (above the white arrows). (× 560.)

FIG. 3. Longitudinal section through stem apex passing to the reproductive state: The youngest leaf-primordium and its provascular strand are at much the same stage of development as those in Pl. VII, Fig. 1. The apex, however, is now strongly arched. (× 260.)

FIG. 4. Longitudinal section through an inflorescence rudiment. The apical meristem extends down the flanks of the rudiment as the peripheral meristem (indicated by white arrows). The provascular cylinder can be seen as meristematic strands extending from the peripheral meristem towards the base of the rudiment (indicated by black arrows). (× 240.)

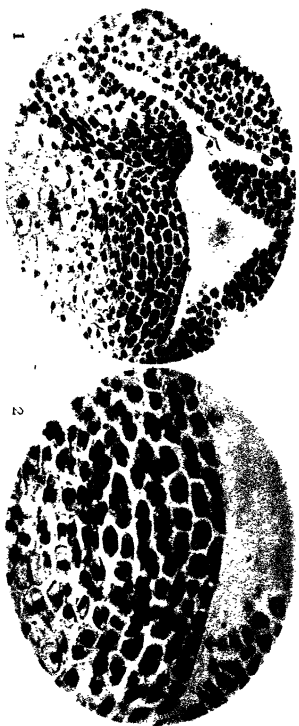
FIGS. 5-8. Longitudinal sections through successively older capitula. 5. At the stage when the involucre bracts are forming. 6. At the stage when flower primordia are beginning to form. The origin of the flower-traces by splitting of the peripheral meristem can be seen. 7. All flower primordia have been formed; floral organs are differentiating. The 'collar' is seen as a circular spot on either side of the peduncle at the level of the involucre. 8. The flower rudiments are more advanced. (× 50.)

FIGS. 9-11. Longitudinal sections through successively older involucre bracts. For explanation see Text-fig. 1. In Fig. 9 the arrows indicate the provascular meristem; in Fig. 10 the origin of the bract trace is indicated by an arrow, and of the gap by an X. (× 360.)

FIG. 12. Longitudinal section through the receptacle. The dark cells are the peripheral meristem, which is giving rise to a succession of flower primordia. From the level of the youngest primordium a provascular strand extends downwards to the inside of the peripheral meristem, from which it is separated by more mature cells. (× 360.)

FIG. 13. Longitudinal section through a flower rudiment. The single vascular strand which enters the rudiment divides into dorsal and ventral 'carpellary' strands. (× 220.)





PHILIPSON - *BELLIS PERENNIS*.

Huth coll.





# The Inhibitory Action of Antibiotics on Plant Pathogenic Bacteria and Fungi

BY

K. GILLIVER

(Sir William Dunn School of Pathology, Oxford)

With four Tables in the Text

**E**XAMPLES of antagonism between soil micro-organisms and plant pathogens have been recognized for many years, and in some instances biological methods of disease control have been elaborated. In recent years specific antibiotic substances have been isolated from culture filtrates of fungi, bacteria, and Actinomycetes, and some of them have been shown to have an antagonistic action against causal organisms of plant diseases. It is possible that certain antibiotics may be of practical use in plant-disease control. The object of this survey was to test under standardized and comparable conditions the action of as many as possible of the antibiotics now available in a highly purified state on a representative selection of plant pathogens.

## PLANT PATHOGENS TESTED

Representatives of as many systematic groups as possible were chosen (Table I). Some organisms have a wide host range, others are specific to one host; they were selected to include a variety of hosts attacked and types of

TABLE I  
*Organisms tested*

Bacteria, gram positive.	Disease produced.
<i>Bacillus subtilis</i> Cohn em. Prazm.	Nil.
<i>Bs. polymyxa</i> (Prazm.) Migula	Gummy rot, potato tubers.
<i>Corynebacterium michiganense</i> (E. F. Sm.) Jens.	Tomato canker.
<i>C. sepeidonicum</i> (Spieck. & Kotthof) Burkholder	Bacterial ring rot, potato.
<i>Leuconostoc</i> sp.	Contaminant in flax retting tanks.
Bacteria, gram negative.	
<i>Bacterium tumefaciens</i> E. F. Sm. & Towns.	Crown gall.
<i>B. aroideae</i> (Towns.) Stapp	Soft rots, ubiquitous.
<i>B. carotovorum</i> (L. R. Jones) Lehm.	Soft rots, ubiquitous.
<i>Pseudomonas marginalis</i> (N.A. Brown) Stapp	Lettuce marginal spot.
<i>Ps. syringae</i> van Hall	Lilac wilt.
<i>Xanthomonas begoniae</i> (Takimota) Dowson	Begonia wilt.
<i>X. campestris</i> (Pamm.) Dowson	Black rot, Crucifers.
<i>X. malvacearum</i> (E. F. Sm.) Dowson	Angular leaf-spot, cotton.
Actinomycetes.	
<i>Actinomyces scabies</i> (Thaxt.) Guss.	Potato Scab.
Phycomycetes.	
<i>Pythium ultimum</i> Trow <sup>1</sup>	Watery wound rot, potato.
<i>Phytophthora erythroseptica</i> Pethybr. <sup>1</sup>	Pink rot, potatoes.

<sup>1</sup> These fungi either did not spore in culture or were more conveniently handled as non-sporing fungi.

TABLE I (continued)

Ascomycetes.	Disease produced.
<i>Botrytis cinerea</i> Fr.	Grey mould.
<i>Byssosclamyces fulva</i> Olliver & G. Sm.	Heat-resistant contaminant of processed fruit.
<i>Claviceps purpurea</i> (Fr.) Tul. <sup>1</sup>	Ergot.
<i>Fusarium avenaceum</i> (Fr.) Sacc.	Foot rot, cereals.
<i>F. culmorum</i> W. G. Sm.	Foot rot, cereals.
<i>Penicillium digitatum</i> Sacc.	Storage rot, oranges.
<i>P. expansum</i> Thom	Storage rot, apples.
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary <sup>1</sup>	Storage rots, potatoes, carrots, &c.
Basidiomycetes.	
<i>Rhizoctonia crocorum</i> Fr. <sup>1</sup>	Purple root rot, carrots, &c.
<i>R. solani</i> Kuhn <sup>1</sup>	Black scurf, potatoes.
<i>Stereum purpureum</i> (Pers. ex Fr.) Fr. <sup>1</sup>	Silver leaf, plums.
Fungi Imperfecti.	
<i>Alternaria citri</i> Pierce	Storage rot, citrus fruit.
<i>Cladosporium herbarum</i> Fr.	Black mould, wheat.
<i>Gloeosporium musarum</i> Ck. & Masee	Ripe fruit rot, bananas.
<i>Myrothecium roridum</i> Tode ex Fr.	Antirrhinum wilt.
<i>Trichothecium roseum</i> Fr.	Storage rot, apples.
<i>Verticillium dahliae</i> Kleb.	Wilts, tomato, raspberry, &c.

disease produced. Selection was limited since only pathogens which can be grown in pure culture could be tested. Powdery mildews, rusts, and other obligate parasites had therefore to be excluded.

The thirteen antibiotic substances tested are listed in Table II.

TABLE II

## Antibiotics tested

Antibiotic.	Produced by.	Reference.
Claviformin	<i>Penicillium claviforme</i>	Chain et al. 1942.
(Synonyms: clavacin, patulin, clavatin, expansine)		
Proactinomycin	<i>Proactinomyces</i> sp.	Gardner and Chain 1942.
Penicillic acid	<i>Penicillium puberulum</i>	Birkinshaw et al. 1936.
	<i>P. cyclopium</i>	
Aspergillilic acid	<i>Aspergillus flavus</i>	Jones et al. 1943.
Gliotoxin	<i>Gliocladium fimbriatum</i>	Weindling and Emerson 1936.
Helvolic acid <sup>2</sup>	<i>Aspergillus fumigatus</i> mut. <i>helvola</i>	Chain et al. 1943.
Mycophenolic acid	<i>Penicillium brevi-compactum</i>	Alsberg and Black 1913.
		Clutterbuck et al. 1932.
Penicillin	<i>Penicillium notatum</i>	Abraham et al. 1941.
Tyrothricin	<i>Bacillus brevis</i>	Dubos and Hotchkiss 1941.
Berberine	<i>Berberis</i> sp.	(Berberine is a readily obtainable substance).
Cheiroline	<i>Cheiranthus</i> spp.	Osborn, unpublished.
Spiraea extract	<i>Spiraea</i> sp.	Osborn, unpublished.
Burdock extract	<i>Arctium</i> sp.	Osborn, unpublished.

A series of tests with proflavine, a chemotherapeutic agent of non-biological origin, was also carried out.

<sup>1</sup> These fungi either did not spore in culture or were more conveniently handled as non-sporing fungi.

<sup>2</sup> Helvolic acid and gliotoxin are both produced by this mould, and the mixture of these substances which Waksman isolated was called by him fumigacin. 'He now wishes to use the name fumigacin for only the helvolic-acid component of the mixture. (Waksman, 1944.)

## EXPERIMENTAL

Preliminary tests on agar plates having yielded only qualitative results which could not always be satisfactorily repeated, it was decided to use only the serial dilution test method. This method gave quantitative results, the most striking of which were successfully repeated some months later.

*Media.* Stock cultures of fungi were kept on Dox's agar. Dilution tests with fungi were carried out in a modified Sabouraud broth containing per litre 10 gr. peptone, 40 gr. maltose, and 26 c.c. malt extract. The reaction was adjusted to pH 6.8 before autoclaving.

Stock cultures of the bacteria and of *Actinomyces scabies* were kept on agar containing per litre 4 gr. Lemco, 10 gr. peptone, 5 gr. sodium chloride, and 10 gr. glucose, with the reaction adjusted to pH 6.8. A similar glucose Lemco broth was used in the dilution tests.

*Antibiotic solutions.* With the exception of gliotoxin and tyrothricin, all antibiotics were made up as a 1/500 aqueous solution. A saturated solution of gliotoxin with a concentration of 1/14,000 was used. Tyrothricin was dissolved in 10 per. cent. alcohol. These solutions were sterilized by passage through a sintered glass filter.

*Procedure for bacterial dilution tests.*

6 in.  $\times$   $\frac{5}{8}$  in. test-tubes, previously plugged and sterilized, were used throughout. Into each tube was put 4.5 c.c. sterile broth and 0.5 c.c. sterile solution of the antibiotic which had been serially diluted so that the concentration in the tubes was progressively halved. The range of concentrations in the first nine tubes of each test was from 1/5,000 to 1/1,280,000. The tenth tube was a control in which 0.5 c.c. sterile distilled water had been added to the broth. In testing gliotoxin, 1.5 c.c. of the solution of the antibiotic was added to 4.5 c.c. of broth in each tube and the range of concentrations was from 1/56,000 to 1/14,336,000. The addition of antibiotic at the strongest concentration tested in no instance altered the reaction of either kind of broth.

*Cultures and inoculation.* Twenty-four-hour cultures were used throughout. Most cultures were diluted to 1/1,000 before use, but the slowly growing *Corynebacterium* spp. and *Xanthomonas* spp. were used without dilution. One drop of the diluted or undiluted culture was introduced into each tube by Pasteur pipette. *Actinomyces scabies* was inoculated from agar slopes, a moistened loop rubbed on the colonies transferring a sufficient though invisible inoculum.

*Incubation.* All tests were incubated at 24° C. The results were read at 2 to 3 days in the case of the more quickly growing bacteria. There was occasionally growth in one tube lower in the series at 3 days, but after that there was no further development if the tubes were incubated for another day. Tests with *Corynebacterium* spp. and *Xanthomonas* spp. and with *Actinomyces scabies* were read at 6 to 7 days. The end point in any test was the highest dilution of antibiotic at which there was no growth of the organism under test. This is expressed in the Tables as the titre.

None of the bacterial dilution tests were duplicated as a routine, but confirmation of high titres was always carried out in duplicate. That there should be small discrepancies in duplicated or repeated tests which were incubated for from 3 to 7 days is almost inevitable, but there was rarely a discrepancy of more than one tube in duplicated tests, and repeated tests never showed an end point more than one tube higher or lower in the series than in the original tests. The lowest figure is given in the Table whenever there was a discrepancy.

No attempts were made to distinguish between inhibition of growth and killing of any of the bacteria and fungi tested.

#### *Procedure for testing fungi.*

These tests were carried out in 6 in.  $\times$  1 in. boiling-tubes. Six c.c. of broth and 0.6 c.c. of the appropriate antibiotic solution were introduced into each tube. A four-fold dilution series was used and the range covered was again approximately 1/5,000 to 1/1,280,000. The sixth tube in the test was a control in which 0.6 c.c. sterile distilled water had been added to the broth. In the case of gliotoxin the amount added to each tube was 1.5 c.c. and the range covered was 1/70,000 to 1/17,920,000.

*Inoculation.* Spore suspensions were made by shaking the cultures with sterile distilled water; three drops of the suspension were introduced by Pasteur pipette into each tube. Inoculation with the non-sporing fungi was effected by introducing small and approximately equal pieces of the agar slope cultures, with some agar adhering, into the tubes. These pieces fell to the bottom of the liquid upon shaking.

*Incubation.* The sporing fungi had generally formed complete and sporing mats on non-inhibiting media in 7 days. Tubes which showed no growth were incubated at 24° C. for a further 7 days, but only very slight or abnormal growth ever developed. The 'non-sporing' fungi were incubated until the mycelium had reached the surface and was forming a mat there. This took about 6 days for *Pythium ultimum* in the control tubes and up to 3 weeks for *Rhizoctonia crocorum*. Tubes which showed inhibition were incubated for 3 weeks but growth very rarely began after 14 days.

#### *Persistence of antibiotic activity during incubation of the dilution tests with fungi.*

Samples of the culture fluids in the tubes containing test substances at 1/5,000 concentration were aseptically removed at intervals and were placed in open-ended cylinders on nutrient agar plates which had been seeded with a 1/1000 of an overnight broth culture of *Staphylococcus aureus* N.C.T.C. 6571. After incubation overnight at 37° C. the diameter of the zone round the cylinder where the growth of the bacterium had been inhibited gave an indication of the activity of the culture fluid. Berberine and proflavine showed only slight traces of activity against *S. aureus* at 2 days. Penicillin and proactinomycin showed considerable activity over the period required to indicate that there was no inhibition of the sporing fungi. An additional

0.3 c.c. of penicillin solutions was added at 4 days in the testing of some non-sporing fungi, but some growth had already occurred of all except *Pythium ultimum*. The activity of penicillic acid disappeared after 6 to 10 days, but *Alternaria citri* was the only fungus which grew after this period. For this species, therefore, and in the four examples quoted below, the test was valueless. Mycophenolic acid became inactive at 9 days and *Fusarium avenaceum* and *Pythium ultimum* grew after this period. Growth had occurred in the other uninhibited fungi before 9 days. Gliotoxin became inactive and growth began at 6 days in the case of *Pythium ultimum* and *Rhizoctonia solani*; activity remained high for at least 14 days in the case of the four fungi which were completely inhibited. The activity of aspergillic acid was only slightly reduced at 12 days, and *Fusarium avenaceum*, the only fungus to grow in a concentration of 1/5,000, did so at 2 days.

The activity of tyrothricin could not be tested in this way because it does not diffuse through agar.

#### *Reproducibility of results.*

All dilution tests with fungi were duplicated. The sporing fungi sometimes developed more quickly in one of the duplicate rows, but by 7 days, when the results were read, the discrepancy was confined to those tubes showing reduced growth compared with the controls. Occasional tubes containing a small abnormal colony were regarded as being completely inhibited, but generally inhibition, if it occurred, was quite complete, and the medium devoid of growth.

The 'non-sporing' fungi gave less clear-cut results and a much longer incubation was needed before discrepancies were reduced. If no hyphae grew out from the inoculum, inhibition was complete. Occasionally some hyphae grew a few millimetres and then collapsed or developed no further; this was also classed as complete inhibition. If the growth was very much slower than in the controls and resulted in less mycelium at the end of 3 weeks the result was classed as partial inhibition and is shown as such in the Table. As with the sporing fungi, discrepancies usually occurred, if at all, in the dilution at which there was partial inhibition. When the dilution giving complete inhibition was in doubt the lower figure is given in the Table.

Repetitions of the tests with fungi inhibited by claviformin, gliotoxin, and cheirolone were made. Most results were confirmed exactly, and the others varied by only one dilution higher or lower than the original results. The lower figures are given in the Table.

## RESULTS

Detailed results are given in Tables III and IV.

### *Bacteria*

Gram-positive bacteria were more sensitive than gram-negative bacteria to proactinomycin, which inhibited no gram-negative species, and to

TABLE III

Dilution giving complete inhibition.	Proactino-mycin.	Penicillic acid.	Aspergillie acid.	Claviformin.	Helvolic acid.	Chetrolin.	Mycophenolic acid.	Penicillin.	Tyrosine.	Burdock.	Spiraea.	Proflavine.
<i>Bacillus subtilis</i>	80,000	40,000	80,000	80,000*	—	40,000	20,000*	0.04	1,280,000	0	5,000*	80,000
<i>Bs. polymyxa</i>	160,000	20,000	80,000*	80,000*	—	20,000	—	1*	0	0	—	160,000
<i>Cornebacterium michiganense</i>	160,000	10,000	80,000	20,000	—	40,000	320,000	0.25	5,120,000	0	0	80,000
<i>C. tepidonotum</i>	160,000*	20,000*	320,000*	80,000	160,000*	80,000	160,000	0.08	10,240,000	5,000	10,000	160,000
<i>Leuconostoc</i> sp.	0	80,000*	320,000*	160,000	—	40,000	0	8	80,000*	0	40,000	80,000
<i>Bacterium tumefaciens</i>	0	40,000	80,000*	160,000	—	80,000*	0	4	80,000	0	5,000*	160,000
<i>B. caridiae</i>	0	160,000	80,000	160,000*	5,000*	80,000	0	4	10,000	0	20,000	0
<i>B. carotocorum</i>	0	160,000	80,000	320,000	0	40,000	0	0	40,000	—	—	10,000*
<i>Pseudomonas marginalis</i>	0	5,000	40,000	40,000	20,000†	0	0	0	0	0	0	0
<i>P. syringae</i>	0	40,000	80,000	20,000	40,000	20,000*	10,000*	0	5,000*	0	0	160,000
<i>Xanthomonas begoniae</i>	0	—	80,000	160,000*	—	20,000*	0	4	40,000	0	10,000	—
<i>X. campestris</i>	0	320,000	160,000	160,000	40,000†	10,000*	0	8	80,000	0	0	20,000
<i>X. maltvarum</i>	0	1,280,000	80,000	320,000	20,000	20,000	0	0	160,000	0	10,000*	40,000
<i>Actinomyces scabies</i>	40,000	40,000	160,000*	160,000	—	20,000	10,000	1*	80,000	0	20,000*	640,000

— = no test made

0 = no inhibition at highest concentration tested (generally 1/5,000).

\* = partial inhibition in next tube.

† = partial inhibition only.

‡ = figures showing the minimum number of Oxford units per c.c. which will completely inhibit growth of sensitive species.

mycophenolic acid, penicillin, tyrothricin, helvolic acid, and gliotoxin. The highest titres were obtained with penicillin, tyrothricin, and gliotoxin.

*Xanthomonas malvacearum* gave the highest titre (unconfirmed) with penicillic acid but, with this exception, gram-positive and gram-negative bacteria were equally sensitive to penicillic acid, aspergillic acid, claviformin, cheirolone, berberine, spiraea extract, and proflavine.

*Leuconostoc* sp. was the least sensitive of the gram-positive bacteria, and *Xanthomonas* spp. the most sensitive of the gram-negative bacteria. *Actinomyces scabies* was inhibited by all the active substances tested, but rarely at a very high dilution.

### Fungi

Sporing fungi were much less sensitive to all antibiotics than those which were handled as 'non-sporing' types. Thus *Alternaria citri*, *Botrytis cinerea*, and *Penicillium expansum* were inhibited only by aspergillic acid, at 1/5,000 concentration, while *Pythium ultimum* and *Phytophthora erythroseptica* were inhibited by 6 and 7 substances respectively, sometimes at 1/320,000. *Fusicoccium dahliae* was the most sensitive of the sporing fungi, and behaved very similarly to *Rhizoctonia crocorum* and *R. solani*.

Aspergillic acid and cheirolone inhibited 17 and 16 fungi respectively, with cheirolone inhibiting at much higher dilutions. Claviformin inhibited 10 fungi, but in general inhibited at higher dilutions than the other antibiotics. Proactinomycin, helvolic acid, penicillin, berberine, burdock extract, and proflavine had little or no activity against the fungi tested.

## DISCUSSION

### Claviformin

Anslow, Raistrick, and Smith (1943) gave data for the inhibition of *Pythium ultimum* by claviformin. In view of the fact that they used Czapek-Dox solution, smaller test-tubes, a greater volume of medium, and claviformin sterilized by steaming, there is remarkable agreement between their figures for complete inhibition at 1/500,000 and partial inhibition at 1/1,000,000, and the figures in Table IV. They showed that *P. aphanidermatum* and *P. mammilatum* at 1/400,000 and *P. debaryanum* at 1/500,000 were also completely inhibited. *Phytophthora erythroseptica*, the other phycomycete listed in Table IV, was completely inhibited at 1/320,000, while non-sporing fungi from other systematic groups were less sensitive. Van Luijk (1938) showed that a culture filtrate of *Penicillium expansum* inhibited *Pythium debaryanum* at a dilution of 1 in 1,280.

Waksman and Bugie (1943) showed that *Ceratostomella ulmi*, the causal organism of Dutch Elm disease, is inhibited in nutrient broth by claviformin at 1/40,000. This figure is of the same order as that of the other sensitive fungi in Table IV. Zentmyer (1942) showed that this disease can be retarded by injections of certain organic compounds if it has not become too well

TABLE IV

Dilution giving complete inhibition.	Proactino-mycin.	Penicillic acid.	Aspergillic acid.	Claviformin.	Helvolic acid.	Mycophenic acid.	Penicillin.	Berberine	Chenopline	Tyrosin.	Spiraea.	Burdock.	Glutoxin.	Proflavine.
<i>Alternaria citri</i>	—	0	5,000*	2	—	1	0	0	1	2	—	—	0	0
<i>Botrytis cinerea</i>	—	0	5,000*	0	—	1	0	0	5,000*	1	—	—	0	0
<i>Byssinhamys fulva</i>	0	1	5,000*	1	—	1	0	0	5,000*	1	—	—	0	20,000*
<i>Gladosporium herbarum</i>	0	—	20,000	80,000	—	20,000*	0	2	50,000	2	5,000*	—	70,000	1
<i>Claviceps purpurea</i>	—	1	2	1	0	2	0	0	5,000	2	0	0	1	1
<i>Fusarium avenaceum</i>	—	—	—	1	0	2	0	—	5,000	2	—	—	1	—
<i>F. culmorum</i>	—	1	—	—	—	0	1	0	5,000	2	—	—	1	1
<i>Glaosporium musarum</i>	0	1	5,000*	5,000*	—	0	1	0	20,000	5,000*	—	—	1	1
<i>Myrothecium roridum</i>	0	0	5,000*	2	—	2	0	0	5,000*	2	—	—	0	2
<i>Penicillium digitatum</i>	0	1	5,000*	5,000*	—	2	0	0	20,000	2	—	—	0	1
<i>P. expansum</i>	—	—	5,000	1	0	2	0	0	2	0	—	—	1	1
<i>Phytophthora erythroseptica</i>	0	—	20,000†	320,000	—	20,000*	1	1	320,000	5,000*	320,000	1	70,000†	0
<i>Pythium ultimum</i>	0	5,000	20,000	800,000x	—	2	7,000	0	80,000	2	20,000	1	1	0
<i>Rhizoctonia crocorum</i>	0	2	5,000†	5,000†	—	20,000†	0	2	5,000*	5,000*	1	0	1	1
<i>R. solani</i>	0	1	5,000*	20,000*	—	5,000*	1	0	20,000	20,000*	2	0	1	1
<i>Sclerotinia sclerotium</i>	0	1	20,000	5,000*	—	—	2	0	5,000	20,000*	2	0	70,000	1
<i>Stereum purpureum</i>	0	1	20,000*	20,000	—	80,000*	0	0	20,000*	5,000†	2	1	70,000	5,000
<i>Trichothecium roseum</i>	0	1	5,000*	1	—	1	0	0	20,000	1	0	—	0	5,000
<i>Verticillium dahliae</i>	0	1	5,000*	5,000	—	20,000*	0	5,000	5,000*	5,000*	2	0	1	5,000

— = no test made. 0 = no inhibition or retardation of growth at highest concentration tested, generally 1:5,000 (Glutoxin) 1:70,000).

1 = retardation in 1st tube (1:5,000 except for Glutoxin).

2 = retardation in first 2 tubes.

x = partial inhibition at 1:1,000,000.

\* = partial inhibition in next tube.

† = partial inhibition in next 2 tubes.

‡ = 1:5,000 solution contains 32 Oxford units per c.c.



established in the host. It would be of interest to see if claviformin and gliotoxin would have a beneficial effect *in vivo*.

The data published for the activity of claviformin against plant-pathogenic bacteria are not readily comparable with those in Table III. Anderson (1943) tested 20 species in liquid media and concluded that there was no relation between sensitivity and the gram reaction. *Xanthomonas stewarti* and *X. pruni* were the most sensitive pathogens tested, being completely inhibited at the same dilution as *Staphylococcus aureus*. Chain et al. (1942) showed that claviformin completely inhibited *S. aureus* at 1/160,000, which is the figure given for the two *Xanthomonas* spp. in Table III. Waksman, Bugie, and Riley (1944) used agar media and expressed their results in 'units of activity'. *B. tumefaciens*, *S. aureus*, *Pseudomonas syringae* and *Bacillus subtilis* were inhibited at equal dilutions and *Corynebacterium michiganense* at a higher concentration. In Table III *Ps. syringae* has a low sensitivity, equal to that of *C. michiganense*.

### Gliotoxin

Weindling (1941) reported that 50 per cent. of the hyphae of *Rhizoctonia solani* mounted under sterile conditions in drops of gliotoxin solution were disorganized after 24 hours in a concentration of 1/300,000. The discrepancy between this result and the figure given in Table IV may be explained either by the absence of nutrients in Weindling's tests, or by strain differences in the *Rhizoctonia* cultures, or by the great differences in methods. Waksman and Bugie (1943) showed that *Ceratostomella ulmi* is inhibited in nutrient broth at 1/12,000.

Johnson, Bruce, and Dutcher (1943) showed that gliotoxin has a highly selective action against fungi and has a toxicity approximately two-thirds of that of mercuric chloride. None of the fungi in Table IV were tested, but titres of 1, 10,000 and 1/16,000 were given for other species. As an example of the importance of the medium used in the test, *Epidermiphyton* sp. was stated to be completely inhibited at 1/260,000 on agar and at 1/60,000 in broth.

Waksman and Woodruff (1942) found that *Bacillus subtilis* was inhibited at 1/1,000,000 on agar. Dutcher (1942) stated that on agar media *Staphylococcus aureus* is inhibited at 1/1,000,000 and all the gram-negative bacteria tested at 1/100,000. Johnson, Bruce, and Dutcher (1943), using gliotoxin sterilized by autoclaving, found that *Corynebacterium michiganense* was inhibited at 1/100,000 in glucose broth, and that 10 species of plant-pathogenic bacteria showed delayed growth at 1/1,000.

Discrepancies between these figures may be accounted for by differences in methods and media; in particular autoclaving would probably destroy some of the gliotoxin activity.

### Penicillin

Penicillin shows only very slight activity against fungi. Waksman and Bugie (1943) reported no activity against *Ceratostomella ulmi*.

Waksman, Bugie, and Riley (1944) placed *Bs. subtilis*, *C. michiganense*,

*B. tumefaciens*, and *Ps. syringae* in the same order of sensitivity as in Table III, *Ps. syringae* being only very slightly sensitive, and *B. subtilis* being inhibited at very high dilutions.

Brown and Boyle (1944a) stated that penicillin produced rings of inhibition of equal sizes when tested against *S. aureus* and *Erwinia carnegiana* and that *C. sepedonicum* was also inhibited. The same authors (1944b) claimed to have cured experimental crown galls, caused by *B. tumefaciens*, by applying wool soaked in crude culture filtrates containing 2 to 6 Oxford units of penicillin per c.c., to galls pricked with a sterile needle. Gall tissue was completely destroyed, and normal tissue inside the stem was unaffected. *B. tumefaciens* therefore appears to be inhibited *in vivo* and *in vitro* by the same concentration of penicillin, and if this work can be confirmed it will be encouraging to find that a disease caused by an organism inhibited at a comparatively low titre *in vitro* can apparently be dealt with so simply.

### *Tyrothricin*

Stokes, Peck, and Woodward (1942) found that four ringworm fungi, tested on agar plates, were inhibited at concentrations of 1/5,000 to 1/20,000. These figures are similar to those for some plant pathogenic-fungi.

Dubos and Hotchkiss (1941) showed that the two constituents of tyrothricin, gramicidin and tyrocidine, were both active against gram-positive bacteria at very high dilutions. At lower dilutions tyrocidine also inhibited some gram-negative bacteria, but this activity was greatly reduced in the presence of broth. The proportion of tyrocidine in different samples of tyrothricin varies and that in the tyrothricin used in the present work was not known.

Waksman and Woodruff (1942) stated that *Bs. subtilis* was inhibited by tyrothricin at 1/330,000 on nutrient agar.

From Table III it is evident that tyrothricin inhibits gram-positive plant pathogens at very high dilutions.

Considering the difference in the techniques used, there is close agreement between these earlier figures and those in Tables III and IV. Published figures of the activity of the other antibiotics against plant pathogens are not available.

Claviformin, gliotoxin, penicillin, and tyrothricin may possibly therefore have applications in plant pathology.

Cheiroline and aspergillic acid are active against many pathogenic fungi *in vitro*, and aspergillic acid might also be of use against diseases caused by some gram-negative bacteria. Mycophenolic acid has a highly selective action against plant pathogens and may have limited application *in vivo*. Penicillic acid might be of use against the gram-negative bacteria causing plant diseases. All these substances are so extremely rare, however, that their practical application is doubtful.

Proactinomycin, helvolic acid, berberine, and the extracts of spiraea and burdock are unlikely to be of any significance in plant pathology.

## SUMMARY

The inhibitory powers of 13 antibiotic substances on 33 causal organisms of plant disease have been investigated by the serial dilution test method. Bacteria on the whole were inhibited by more substances and at higher dilution than fungi. The highest titres obtained were for gliotoxin, penicillin, and tyrothricin on certain gram-positive bacteria. Penicillic acid, aspergillic acid, and claviformin inhibited gram-positive and gram-negative bacteria at approximately equal dilutions.

*Phytophthora erythroseptica* and *Pythium ultimum* were the most sensitive fungi. Sporing fungi were much more resistant than 'non-sporing' fungi.

Aspergillic acid and cheiroline inhibited almost all the fungi tested; claviformin inhibited fewer fungi, but at rather higher dilutions.

Of the substances tested, claviformin, gliotoxin, penicillin, tyrothricin, cheiroline, and aspergillic acid might be useful in the control of plant diseases caused by certain pathogenic bacteria and fungi.

The survey was suggested by Sir Howard Florey, and the author wishes to offer thanks to him and to Dr. M. A. Jennings of this department for their help and advice. The pathogens were selected and cultures of them were supplied by Professor F. T. Brooks and Dr. Dowson of the Botany School, Cambridge, to whom thanks are also due for advice on cultural methods. The aspergillic acid was made available through the kindness of Dr. Rake, and the author is grateful to Mrs. Osborn for providing the plant materials. The work was carried out during the tenure of a grant from the Agricultural Research Council.

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# Note on a *Ulothrix* from a Cheshire Brine Pit

BY

V. J. CHAPMAN

(*Botany School, Cambridge*)

With seven Figures in the Text

CAREFUL examination of some material which had been collected from a brine pit in Cheshire and sent to the writer by Mr. F. Burke showed it to be a *Ulothrix*, but it exhibited some differences which distinguished it from the species so far known. The habitat itself is of interest since it is one of the inland saline areas of this country. It is a disused well from which brine was formerly pumped and is situated about two miles south of Malpas. Further details and a photograph of the pit are given in a brief note by Mr. Burke (1942). In the absence of any consecutive records it is probable that the amount of salt in the medium varies throughout the year, but the habitat is essentially saline. The alga itself was found round the edge of the pit on the damp mud and also floating in the small pools formed by cattle hoof-marks. The salinity of the water in the pit itself is very high, much higher than in other brine pits observed by Mr. Burke. It normally ranges from 9.1 to 9.3 per cent., though after periods of much rain it may fall to 7.9 per cent. There are no data about variations in the salinity of the small pools and the soil except for a single note by Mr. Burke that when the alga was first found in 1944 the salinity of the small pool in which it occurred was 3.72 per cent. The present writer has, however, analysed a sample of mud, which was collected in the late summer of 1944, from the edge of another brine pit (almost certainly of much lower salinity) in Cheshire. Here the chloride content, expressed as a percentage of the air-dry soil, was 0.09 per cent. and the moisture of the air-dry soil was 6 per cent., so that it would be even lower in the total soil water of the freshly collected soil. It is doubtful, therefore, whether in the spring, after the winter rains, the salinity would exceed that of the sea (3.3 per cent.).

One of the difficulties of the identification of any of the maritime species of *Ulothrix* is the lack of adequate illustrations. Most of the species are based upon Wille's study (1900), whilst some further information can be obtained from Hazen's (1902) paper. Both these taxonomic papers are illustrated, but the scale of the drawings is not such as to bring out the details. The written accounts of Wille are, however, very complete. More recently, Carter in her study (1933) of two salt-marshes found several of the species described by Wille and her illustrations are more helpful. It is still evident that there is a great need for a monograph on the genus with adequate illustrations; the

lack of such a monograph was felt acutely in the study of this Cheshire material.

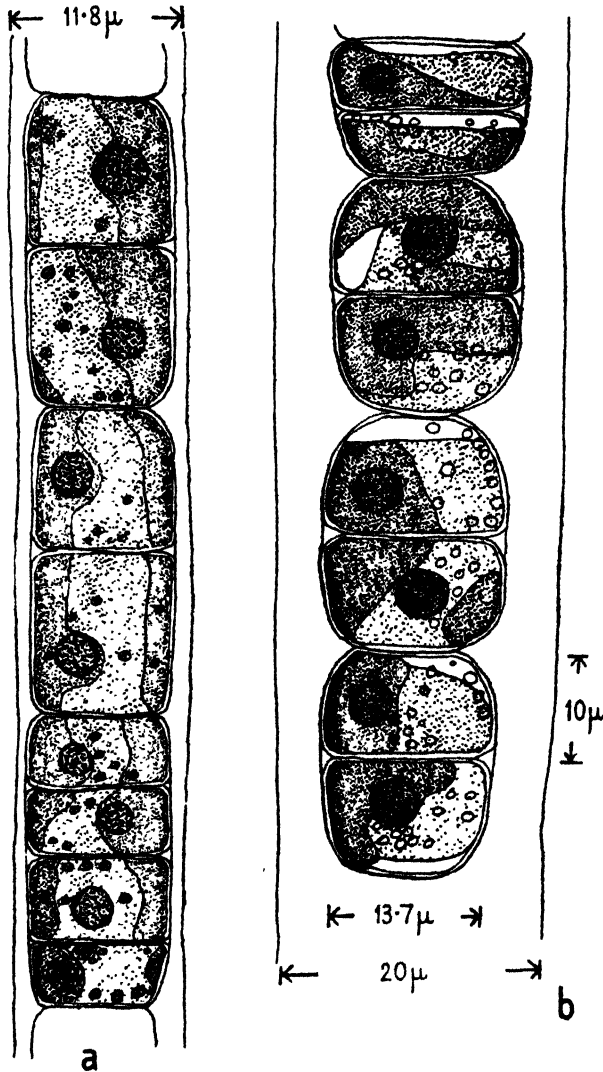


FIG. 1. *a*, Filament showing paired arrangement of cells. The basal pair are in process of further subdivision. *b*, Another filament, shortly before zoospore formation.

The filaments of this form of *Ulothrix* ranged in width from 8 to  $23\mu$ , the commonest measurement being about  $16\mu$  (Figs. 1 and 2), whilst the cells varied from 10 to  $14\mu$  in breadth. In length they ranged from 10 to  $14.6\mu$  and were  $\frac{1}{2}$  to  $1\frac{1}{4}$  times as long as broad. The great majority of the cells were shorter than broad and the commonest ratio appeared to be 5 : 7. The most striking feature about the cells was the tendency for them to be arranged in pairs or sometimes even in fours (Figs. 1 and 2). Even when the pair arrange-

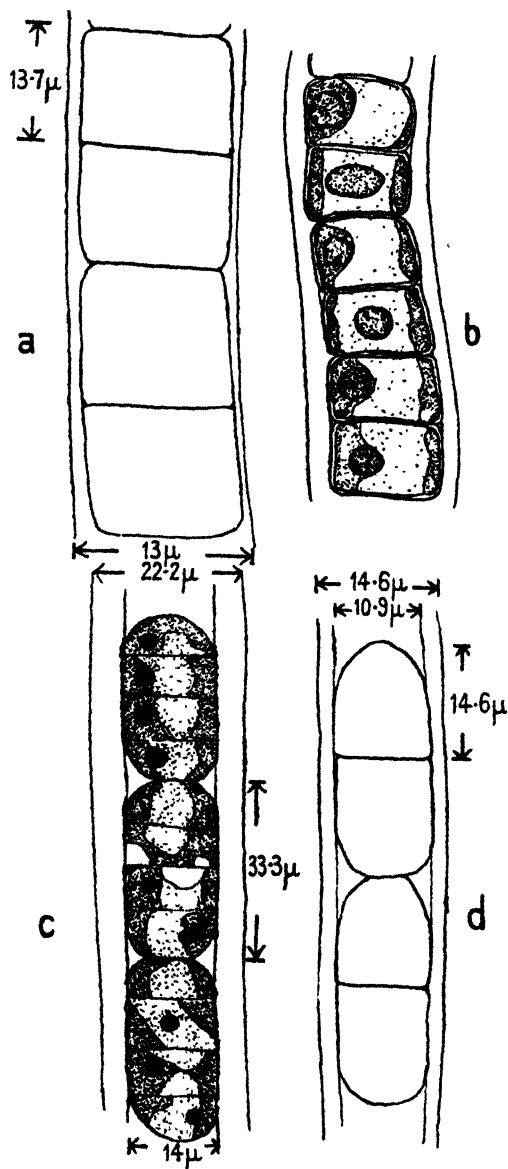


FIG. 2. *a*, Portion of a young filament. *b*, Another young filament. The paired arrangement is not so obvious. *c*, Pairs of cells dividing. Note inner edge of sheath. *d*, Filament showing inner edge of sheath.

ment was not immediately obvious, examination under an oil-immersion lens showed that nevertheless it did exist. In some filaments it is extremely obvious (Figs. 1, *b*; Fig. 2, *c*, *d*; Fig. 6, *e*), whereas in others, especially younger filaments, it is less so (Fig. 2, *b*). The outer wall varies in thickness from  $2.5$  to  $8.2\mu$ , the older filaments having the thicker walls (Fig. 1, *b*; Fig. 2, *c*).

There is some evidence that the wall is composed of more than one layer, and in some of the filaments an inner edge could be discerned where two cells of a pair joined each other (Fig. 2, *c*, *d*; Fig. 6, *e*). Each pair of cells also appeared to be contained within a common envelope, which presumably

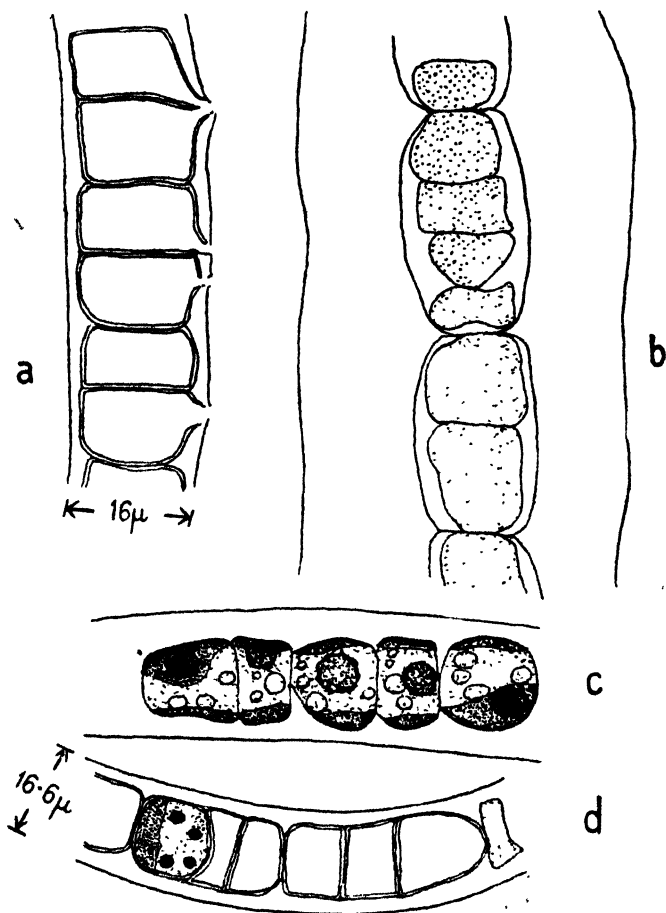


FIG. 3. *a*, Empty cells after escape of zoospores. *b*, Filament stained with iodine. The isolation of the groups is clear. *c*, Filament stained with iodine to show pyrenoids and fat globules. *d*, Groups of cells after escape of zoospores.

represents the wall of the original parent mother cell. This condition is illustrated in Fig. 3, *b*, and Fig. 6, *e*. In addition to the remains of the parent envelope each individual cell possesses its own membrane. This feature can be clearly seen with the aid of an oil-immersion lens (Figs. 1 *a*, and 6, *e*). The development and retention of the cells in pairs can also be observed extremely well in filaments that have discharged their swimmers, and under certain conditions it can be made to show up by staining with iodine (Fig. 3, *b*). Sometimes three cells were found in a group instead of two or four;



in such a case one of the original daughter cells has failed to divide. It would seem that there may, therefore, be three envelopes: (a) the main outer sheath, (b) the parent envelope, and (c) the cell wall of the individual cells.

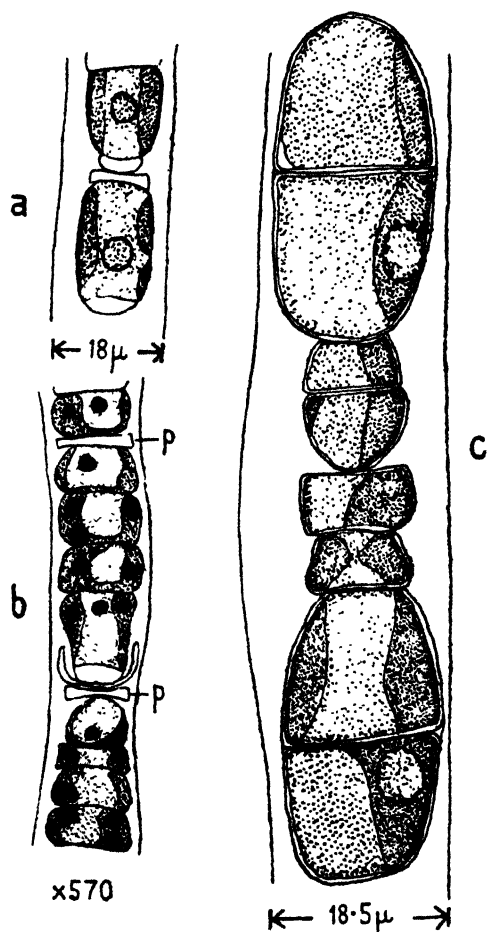


FIG. 4. *a*, Normal and dying cells. *b*, Group of four cells that have originated from a single mother cell and are segregated by cellulose plugs (*p*). *Ulothrix flacca* (after Wille). *c*, Normal and dying cells.

The chloroplast is band-like and under normal conditions it occupies the whole length of the cell. Under unfavourable conditions it commences to disintegrate and shows signs of vacuolation. The banded chloroplast does not extend completely round the cell, and under vegetative conditions there is one large conspicuous pyrenoid. Round the edge of the pyrenoid small granules could be observed, and as these stained with iodine they are probably composed of a starchy material. During reproduction the pyrenoid divides and the same number of daughter pyrenoids are formed as there will be swimmers. The cells also contain globules of fat (Fig. 1; Fig. 3, *c*). No basal

cells were found among the adult plants, but in young plants it was clear that the basal cells contained a chloroplast and were longer than broad (Fig. 6, *c*, *d*; Fig. 7, *h-j*). As in most species of *Ulothrix* some cells fail to develop properly: these can be recognized by their smaller size and darker contents (Fig. 4). Ultimately these cells die and may then become compressed, but no examples were found of extreme compression such as is figured by Wille (1900) for some of the species.

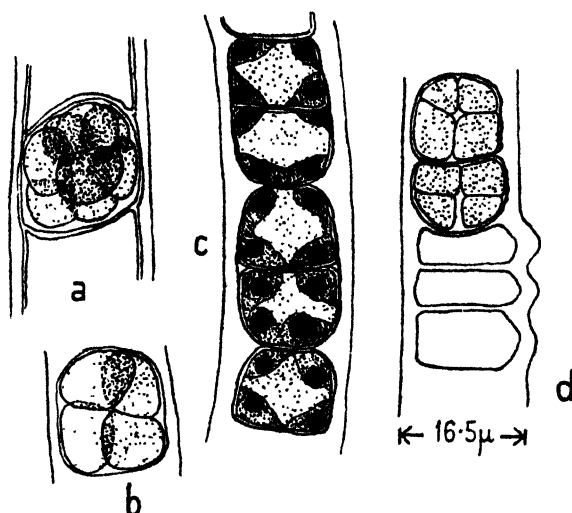


FIG. 5. *a*, Cell with eight macrozoospores. *b*, Cell with four macrozoospores. *c*, Cells at zoospore formation, each with four pyrenoids. *d*, Zoospore formation and empty cells.

At the time the material was received (early March) zoospore formation was actively proceeding. Normally four zoospores are formed per cell (Fig. 5, *b*, *c*), but examples with eight were also to be found (Fig. 5, *a*). The zoospores are liberated into a vesicle through a pore in the side of the cell (Fig. 7, *a*). They are generally oval, though some were observed that were more pyriform in shape (Fig. 6, *a*). Each zoospore contained a basin-shaped chloroplast, a pyrenoid and eye-spot, and possessed four cilia. After liberation the four swimmers swim about for a short time, after which they settle down and round off (Fig. 6, *b*), when one or more vacuoles become apparent. On germination they give rise to new plants, but in the young state the paired arrangement of the cells is not always apparent (Fig. 6, *c*).

The zoospores described above must be regarded as macrozoospores, because when the material had been kept for some days in tap-water some of the filaments were in process of division, and appeared to be producing microzoospores. Very few of the motile swimmers were seen and no cases of fusion were encountered. In spite of this negative evidence these smaller swimmers may have been gametes, but it is usual for a greater number to be produced per cell. Here four seemed to be the most frequent complement for any one cell (Fig. 7, *c*). Apart from reproduction by macro- and

micro(?)zoospores the threads can also perpetuate themselves by fragmentation (Fig. 7, *b*). When this occurs groups of 2, 3, or 4 cells become separated by dead cells, and it is abundantly clear that these units are derived from one or two original mother cells.

The early stages in germination of the macrozoospores could be followed by allowing the zoospores to settle on a microscope slide. These stages are

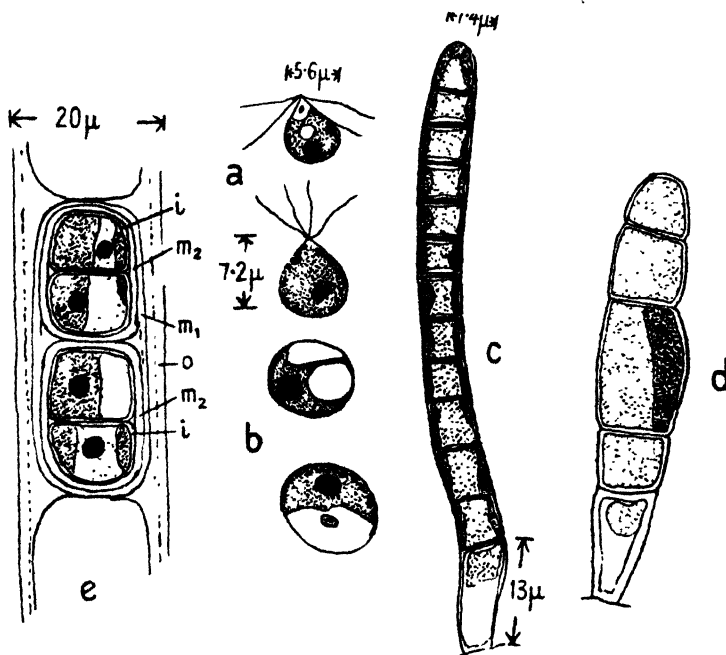


FIG. 6. *a*, Macrozoospores. *b*, Macrozoospores after settling. *c*, *d*, Young plants. *e*, Portion of filament showing the various sheaths after being subjected to desiccation. *m*<sub>1</sub> = first mother cell sheath; *m*<sub>2</sub> = second mother cell sheath; *i* = sheath of existing cells; *o* = outer sheath of filament.

illustrated in Fig. 7. The rounded zoospore (Fig. 6, *b*) first elongates (Fig. 7, *d*) and then cuts off a cell towards one end (Fig. 7, *e*). This small cell is the future attachment cell. It either elongates at once (Fig. 7, *f*) or remains small (Fig. 7, *g*) until the other cell has divided again. Eventually the basal cell becomes longer than broad (Fig. 7, *h*, *i*). In some of the young plants the paired arrangement of the cells may become obvious at an early stage (Fig. 7, *j*).

Like other species of *Ulothrix* the alga is vernal, as the following data from Mr. Burke show. It was first found on January 30, 1944, and had disappeared by May 7; in 1945 it was found on February 25, the first visit of the year.

The plant described above agrees in certain of its characters with *Ulothrix pseudoflaccida* Wille (Wille, 1900). Hazen (1902) and Collins (1909) have stated that they do not consider that there is sufficient justification for

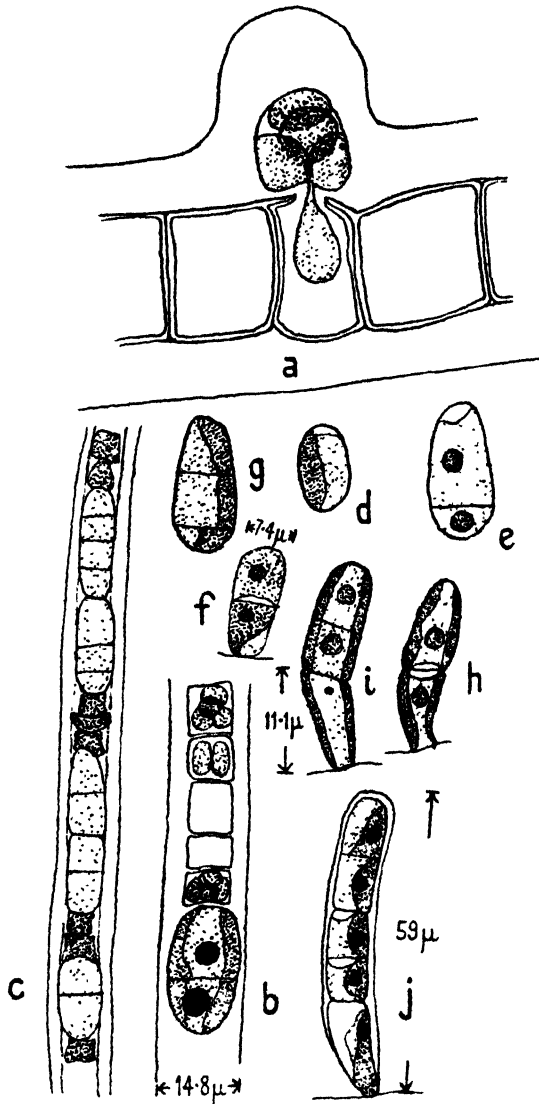


FIG. 7. *a*, Escape of macrozoospores into vesicle. *b*, Micro(?)zoospore formation. *c*, Fragmentation. *d*, 1st stage in germination. *e*, *f*, *h*, 2nd stage in germination. *g*, *i*, 3rd stage in germination. *j*, Young plant showing paired arrangement of cells. (Germination stages are those of macrozoospores.)

distinguishing *U. pseudoflacca* from *U. flacca*. However, Wille's (1900) descriptions indicate that the following characters can be used to separate these two species.

<i>U. flacca</i>	<i>U. pseudoflacca</i>
Filaments 18–30 $\mu$ wide (Broader when producing gametes): 50 $\mu$ (Collins) to 80 $\mu$ (Rosenvinge.)	Filaments 8–22 $\mu$ wide. (Swollen when producing gametes.)
Cells $\frac{1}{2}$ – $\frac{3}{4}$ as long as broad.	Cells $\frac{1}{2}$ as long as broad.
1–3 pyrenoids per cell.	1 pyrenoid per cell.
Rhizoids may be formed from the basal cells.	No rhizoids.

Carter (1933) maintained that there was a distinct difference in size between the two species and she considered their distinction valid. The illustrations of Wille and Hazen show that in *U. flacca* the cells are normally much broader than they are long, whereas in *U. pseudoflacca* this difference is not so pronounced. According to Hazen *U. flacca* is synonymous with *Lyngbya flacca* and *L. carmichaelii* as illustrated by Harvey (1854), plates 300 and 186. Plate 186 (*L. carmichaelii*) would certainly appear to refer to *U. flacca*, but some doubt (felt also by Harvey) appertains to plate 300 (*L. flacca*) because the filaments are shown as branched. *U. flacca* is also illustrated by Dillwyn (1805) in his 'British Confervae' (pl. 49), and in 'English Botany' (1808), 1st ed., pl. 1943. Both these illustrations agree with Wille's account. The present author examined plants of *Lyngbya carmichaelii* collected by Mrs. Griffiths and deposited in the Cambridge Herbarium. The filaments were much wider than those from Cheshire and the cells were much broader than long, nor was there any evidence that cells were in pairs. There would seem little doubt, therefore, that Wille's two species are distinct and that Collins and Hazen were mistaken in their conclusions.

So far as size of filament is concerned our plant agrees more nearly with *U. pseudoflacca*. The principal difference is the arrangement of the cells in pairs, but this is a feature that a careful observer like Wille would scarcely have missed. A comparison of the characters of the two plants brings out the essential differences.

<i>U. pseudoflacca</i>	Cheshire plant
Width of filaments 8–22 $\mu$ .	8–23 $\mu$ .
Ratio, breadth to length $\frac{1}{2}$ to 1.	$\frac{1}{2}$ –1 $\frac{1}{2}$ , usually $\frac{5}{7}$ .
Cells single.	Cells in pairs or fours.
1 pyrenoid.	1 pyrenoid.
Plastid fills length of cell.	Plastid fills length of cell.
4–8 zoospores (commonly 8).	4–8 zoospores (commonly 4).
Zoospores oval—egg-shaped.	Zoospores oval or nearly spherical.
Basal cell only a little longer than broad.	Basal cell longer than broad.
Dead cells considerably extended.	Dead cells not much extended.
No distinct inner edge to sheath.	Inner edge of sheath may be distinct.

Carter (1933) gives two drawings of *U. pseudoflacca* as found on the salt-marshes of Canvey and the Dovey, and in one case the cells are shown arranged in pairs. This feature evidently did not obtrude upon her notice

because no reference is made to it in the text. It is possible, therefore, that she had, in fact, two distinct groups of plants, one of which was perhaps identical with the Cheshire material. Alternatively the occurrence of cells in pairs may have been but infrequent, and *U. pseudoflacca* may occasionally exhibit this feature. Carter's drawings do, however, show that the chloroplasts in her filaments did not occupy the full length of the cells. Further work is necessary in order to ascertain how far this may be a useful diagnostic character. There is also the statement by Wille (1900) that in *U. flacca* the cells are often arranged in groups that have originated from a single cell, and that the groups are separated from each other by a cellulose thickening (plate 1, Fig. 56, reproduced in Fig. 4, *b*). This is similar to the behaviour of the material from Cheshire. On the other hand, it is clear that the filaments of the Cheshire material are narrower than those of *U. flacca* and the cells are relatively longer.

The Cheshire plant, therefore, differs in certain details from both *U. flacca* and *U. pseudoflacca*. Both these species are closely allied, and it does not seem that the differences of the Cheshire material would warrant the establishment of a new species. They are sufficient to justify at least a separate variety until more is known about the genus. On the whole there would seem to be a greater resemblance to *U. pseudoflacca* than to *U. flacca*, and it is therefore proposed to regard the Cheshire material as a variety of *U. pseudoflacca* and it is suggested that it be called var. *salina*.

*Ulothrix pseudoflacca* var. *salina* var. nov.

Filaments 8–22 $\mu$  wide, cells 10–14 $\mu$ ,  $\frac{1}{2}$ –1 $\frac{1}{2}$  as long as broad, commonly arranged in pairs; plastid band-like occupying the length of the cell, with one pyrenoid except when reproducing. Reproduction by 4–8 oval or rounded 4-ciliate macrozoospores per cell, also by microswarmers. Saline brine pit, Malpas, Cheshire.

Co-type deposited in the Cambridge Botany School Herbarium.

I am grateful to Mr. Burke for sending me this material and also to Prof. Fritsch for his advice and the opportunity of discussing the problems with him.

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# **An Apparatus for recording automatically the Course of Bulb Formation, with some Preliminary Observations on Bulb Development in the Onion**

BY

M. HOLDSWORTH AND O. V. S. HEATH

*(Research Institute of Plant Physiology, Imperial College of Science and Technology, London)*

**With five Figures in the Text**

## INTRODUCTION

THE factors controlling flowering and bulbing in onions have been the object of study in this Institute for some years (Heath, 1943, 1943a; Heath and Holdsworth, 1943; Heath and Mathur, 1944; Holdsworth, 1945; Holdsworth and Heath, 1945; Heath, 1945). The main factors controlling bulb formation are day length and temperature. In assessing their effects it is necessary to recognize accurately the first swelling response of the pseudo-stem, and by direct observation swelling cannot be detected with certainty until at least 2 weeks of long-day treatment have been given, for the process begins very slowly. To investigate the period of exposure to long days necessary to initiate bulbing, some means of magnifying the increase in diameter of the pseudo-stem would, therefore, be an advantage.

It was found that under certain conditions onion seedlings could form bulbs after lifting, that is without any external water-supply. Recording the swelling of such plants, which could be mounted dry in the apparatus, presented an easier problem than that of seedlings growing in soil, and a simple instrument for the former purpose was constructed by one of us (O. V. S. H.) from the working parts of a recording hair-hygrograph. This experimental model was successfully used in 1942 to record the bulbing of pulled-up seedlings. Later, an improved apparatus, designed and constructed by the first author, greatly extended the usefulness of the method.

## APPARATUS AND EXPERIMENTAL TECHNIQUE

In the usual forms of recording hygrograph, changes in the length of a bundle of hairs are magnified by a system of two levers. The short arm of the first is connected to the hair and its long arm is curved so that in turning it may roll on a similar curved bar constituting the short arm of the second lever. The long arm of the latter bears the recording pen. For use in recording bulb growth the hair and its supporting frame were removed. The hook normally connecting the hair to the short arm of the first lever was replaced

by a glass square cut from a microscope cover-slip and made fast with sealing-wax. This glass plate was brought to bear on the side of the pseudo-stem of the seedling which lay horizontal and parallel with the axes of the lever system, and was supported in a V-shaped groove formed between a vertical, fixed, glass plate and a pile of almost horizontal loose glass slips. The thickness of the latter could be changed to accommodate seedlings of different diameters. The spring, which in the hygrograph keeps the hair taut, was used to maintain pressure of the lever on the seedling and enabled the instrument to record shrinkage as well as swelling. The record was made on a chart revolving once in 7 days.

Several satisfactory records were obtained, but the apparatus has some disadvantages. For example, in the hair-hygrograph the relation between the movements of the hair and of the pen is expressly made non-linear by means of the curved arms, and the records of bulbing obtained therefore require recalibration. Further, the apparatus is only suitable for use with lifted seedlings since these do not react geotropically. The leaves of plants provided with a water-supply, when mounted horizontally, tend to turn upwards and so cause the plant to roll over and spoil the record.

A satisfactory apparatus on which the plants are mounted vertically and in which the relationship between the movements of the proximal and distal ends of the lever system is very nearly linear is shown in Figs. 1 and 2. The apparatus was mainly constructed of standard 'Meccano' parts and no accurate machining is required for its construction. Satisfactory records of the bulbing both of lifted seedlings and of seedlings growing in soil have been obtained. The provision of a weather-proof housing has enabled a modification of the instrument to be used for plants growing in the open on field plots. In the field the pseudo-stem base was laid bare by excavation, as it was necessary that the bulb should be above soil level in order that the rod might bear on the diameter of maximum expansion. For experiments in the laboratory seedlings were specially grown in glass tubes to make this possible. A small hole was blown in the bottom of a  $2 \times 1$  in. specimen tube; five such tubes were packed with finely sifted soil and buried in a 4-in. pot of soil so that their rims were just level with the surface. On the surface of the soil in each tube four seeds were sown and then the pot filled and levelled off with a further  $\frac{1}{4}$ -in. layer of soil. When germination was complete the seedlings were thinned to leave one in each tube. The seedlings were normally grown in short days (11 hours) until required for use; the soil and tubes were then tapped out of the pot and each plant left in its tube with the base of the pseudo-stem exposed. The laboratory apparatus accommodated four plants at a time. In Fig. 2 is shown the lever system for a single plant. Each tube (A) is held in an adjustable clip (B) clamped to the framework. The adjustable stop (D) is retracted as far as it will go and the plunger (FG) removed before placing the seedling in position. The stop is now adjusted just to touch one side of the seedling, the plunger replaced, and the pen brought to a suitable position on the chart by adjusting the sleeve (H). The zinc water-trough (C), which is



waxed to resist corrosion, is next placed in position, and the water level is maintained at the bottom of the tubes. Roots eventually emerge from the holes in the tubes and hang in the water.

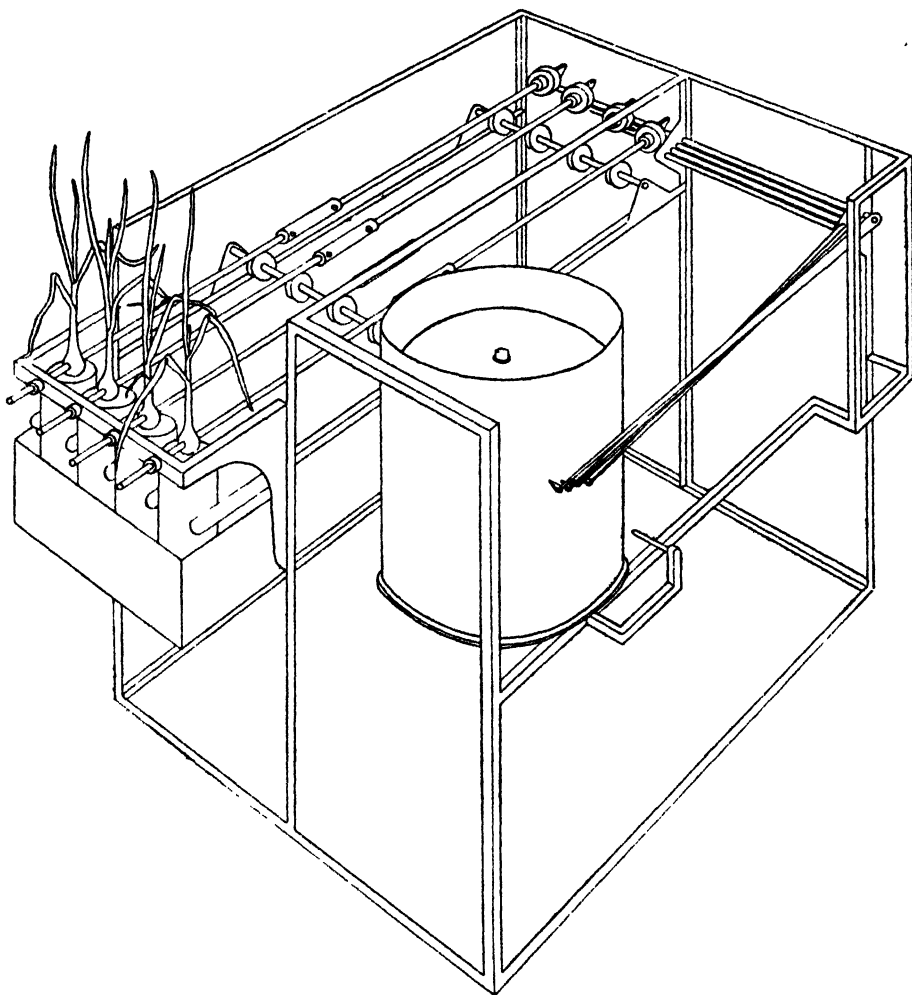


FIG. 1. Perspective view of complete apparatus with four seedlings in position.

As swelling takes place the plunger moves forwards on the idling wheels (K and L) and the glass plate (G) bearing on the pointed lever (J) turns it about (M), thus imparting motion to the lever MN. The drum is from an ordinary thermograph and revolves by clockwork once in 7 days.

The magnification of the system is represented by the ratio of the lengths of the levers (MN and MJ). Since the levers for the four seedlings are of different lengths the magnification is slightly different for each but is about nine times. For small seedlings a greater magnification would be an advantage, but the frictional resistance to motion of the plungers should be kept as small

as possible, as it has been found that a relatively small pressure on one diameter of the bulb suffices to prevent swelling in that direction. When a pen reaches the top of the chart it it can be brought down by retracting the stop (D) and altering the position of the rods in the sleeve (H).

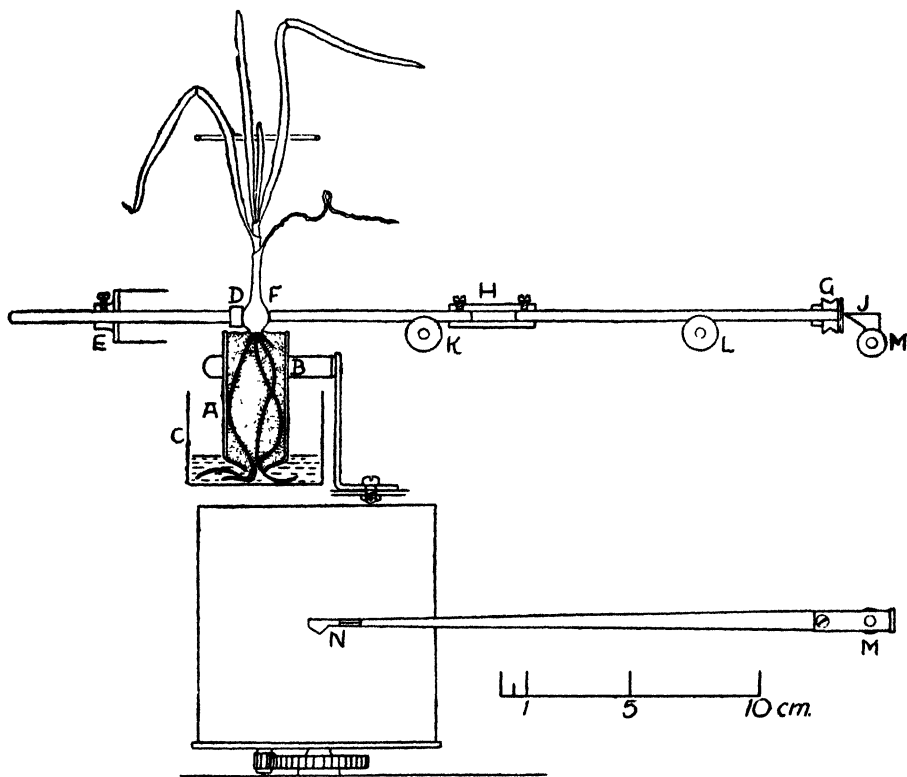
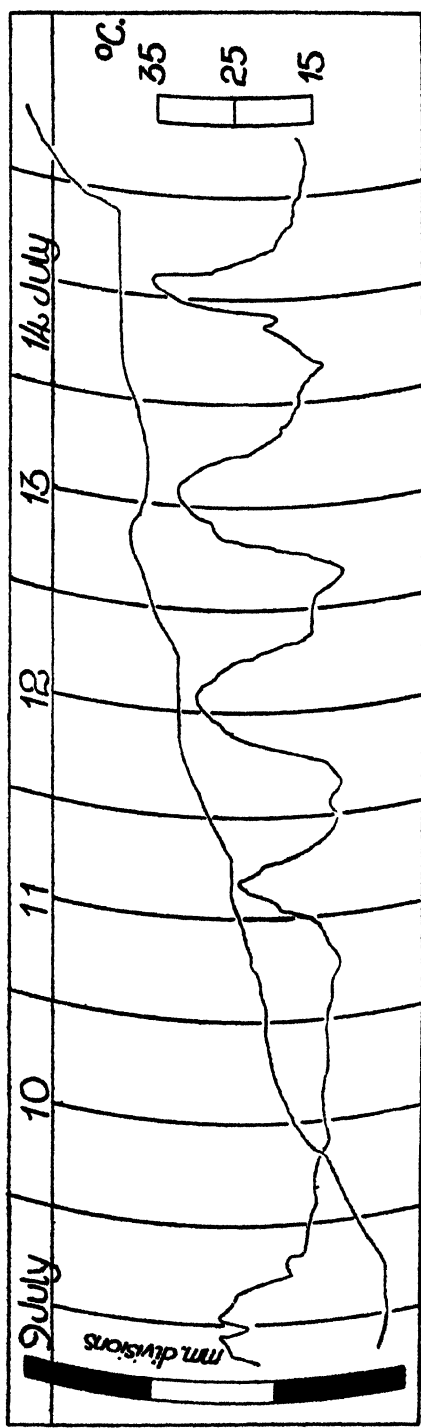
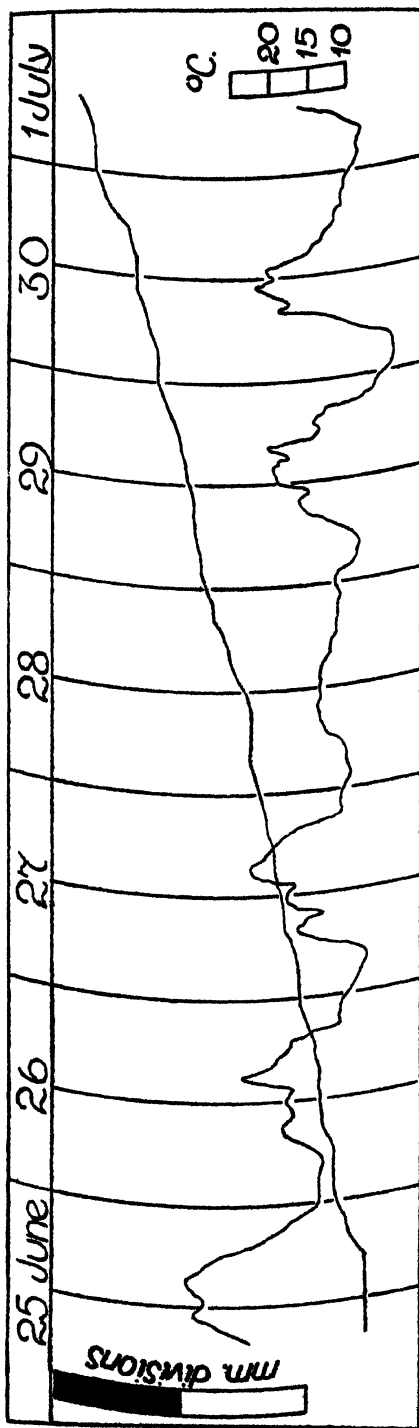


FIG. 2. Lever system for one seedling on the apparatus. (For explanation see text.)

#### SOME BULBING RECORDS OBTAINED

In Fig. 3 are reproduced parts of a bulbing record of a plant (Variety No. 1 of the Horticultural Research Station, Cambridge) growing in the field. This variety is a late type and at outdoor temperatures the process of swelling continued for many weeks. It is seen that the rate of swelling varied considerably on different days and at different and irregular times during the day. Some general conclusions may, however, be derived from these and other records: (1) The rate of swelling is initially small, rapidly increases to a maximum, and slowly declines again with the approach of ripeness. It continues beyond the stage at which leaf emergence ceases and the foliage falls over on to the ground (Holdsworth and Heath, 1945), but is probably complete before all the foliage has withered. (2) On a cool, overcast day swelling continues throughout the 24 hours but is slower during the night corresponding



FIGS. 3a and b. Parts of the record of the swelling of an onion plant in the open (the rising curves); also thermograph records of air temperature.

with the lower temperature. (3) On a hot sunny day the rate of swelling increases for a time after daybreak, but as the strength of the sun increases, slows down and sometimes ceases altogether. When, in the evening, the temperature falls suddenly the rate of swelling suddenly increases, but the new high rate is only maintained for a short time, so that a steep step is produced in the record, as on June 25 and again on July 11 and 12. (4) After a heavy shower a similar steep step was obtained (*vide* on July 14 at 10 p.m.) as a result apparently of increased rate of absorption of water from the soil.

In Fig. 4 is shown part of the record of the bulbing of four seedlings (var. Ebenezer) in the warm greenhouse, together with the greenhouse temperature over the same period. The night temperatures, indicated by dotted lines, are conjectural as the thermograph was placed in a rather warmer position in the greenhouse at night for the purpose of other experiments then in progress. The seedlings were growing in soil in glass tubes and had part of their root system continuously immersed in water. On most days a sudden increase in rate of swelling took place in the evening, some hours before nightfall but at a time when the greenhouse temperature was falling rapidly. An interesting effect is shown in the records on June 20, when sudden falls in temperature occurred both after 11 a.m. and 2.30 p.m., the former during a shower. During that day there are two steep steps at times corresponding to the fall of temperature. In some cases the rate increased slightly during the morning, but in the heat of the day swelling often ceased altogether. Just after bulbing had begun little swelling took place except in the evenings, but as bulbing proceeded the suddenness of the change of rate became less pronounced and swelling did not cease altogether at any time of the day or night. With plants growing under these conditions, the earliest rapid increases in swelling could be detected within two days of the first application of the long-day stimulus.

Fig. 5 is a reproduction of a record obtained with the modified hygrograph from a seedling (Unwin's Reliance) lifted before bulbing had begun. The record has been replotted on a linear scale. The seedling was taken from a field plot, but the apparatus was set up in the warm greenhouse. Immediately after lifting, the seedling wilted and a rapid shrinkage of the pseudo-stem was recorded. The following morning, however, bulbing began suddenly, but the rate of swelling fell off during the day and had practically ceased by nightfall. A high rate of swelling began about the same time each morning following, but the falling off in rate became less pronounced each day; the whole process was completed in about a week. When bulbing began the youngest leaf regained its turgor together with the lower part of the next oldest leaf; the rest of the leaves dried out completely. As bulbing continued the leaves remaining green dried out progressively from the tips downwards, and no green leaf was left when bulbing was complete. It seems therefore that the water necessary for swelling was derived from the leaf blades. Examination of the temperature records shows that rapid swelling accompanied a rapidly rising temperature in the greenhouse.

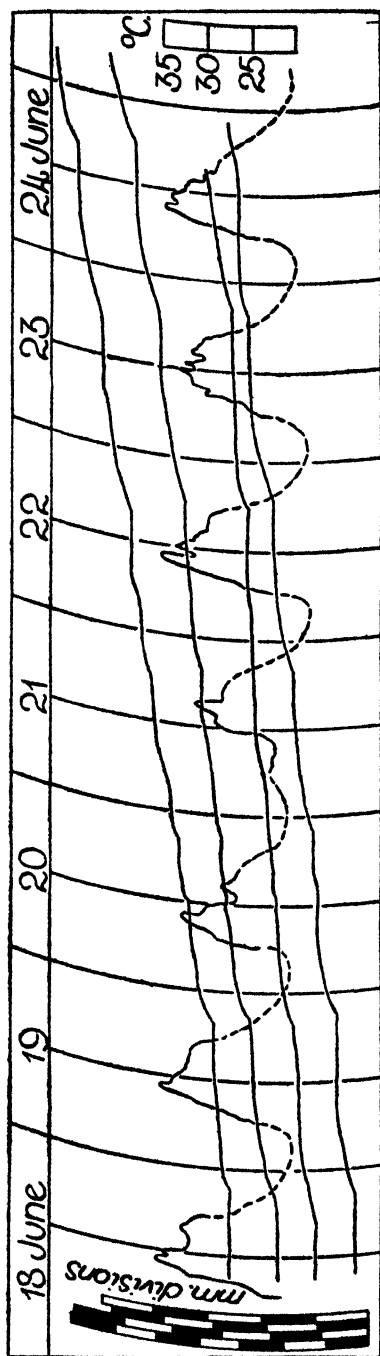


FIG. 4. Records of the swelling of four seedlings in the greenhouse for the third week in long days. An air temperature record has been superimposed, but the night temperatures (broken lines) have been sketched in. The magnification was slightly different for each seedling as shown in the scale of millimetres on the left.

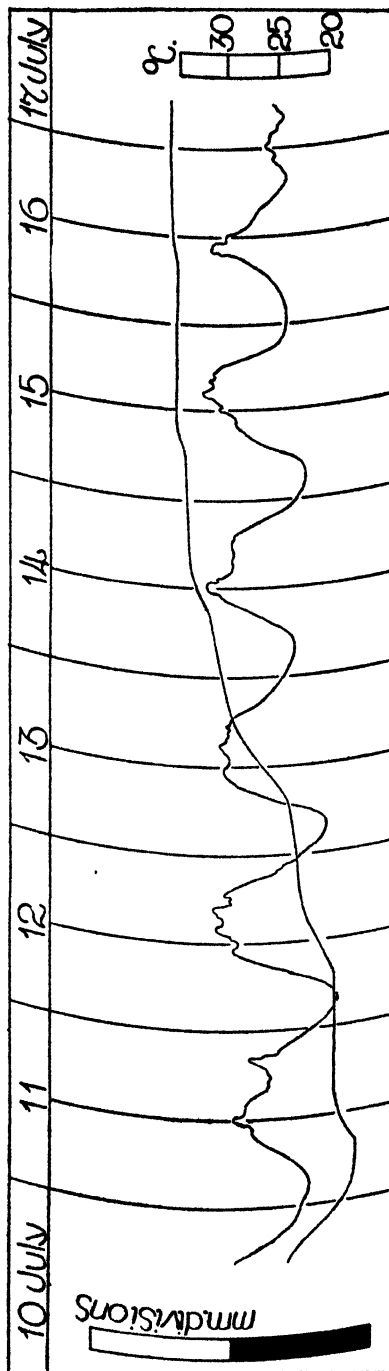


FIG. 5. Record of the swelling of a pulled-up seedling in the warm greenhouse; also thermograph record of air temperature.

It is clear that certain striking differences in relation to temperature distinguish the bulbing records of seedlings: (a) growing in the field, (b) growing in the greenhouse in soil and with their roots in water, or (c) lifted and without water-supply. In the field relatively rapid bulbing occurs at times of moderate temperature whether the temperature is rising, steady, or falling, i.e. in the morning and evening on bright days or throughout the day when cloudy. In the warm greenhouse, with a water-supply, rapid bulbing is mainly associated with a moderate and falling temperature. Finally, in the case of seedlings with no external water-supply, rapid bulbing occurs when the temperature rises but not when it falls. Further investigation is clearly needed before a reason can be assigned for these contrasts, and a full discussion would be premature, but acceleration of swelling by moderately high temperatures would seem to be indicated. Using the apparatus with plants kept under conditions of controlled day length, light intensity, and temperature it has been shown that changes in rate of swelling can be induced by varying the temperature. Records of the same form as those from the greenhouse have in this way been obtained both with lifted seedlings and with seedlings growing in soil, and in each case the effects of changing temperature were of the same kind as those described above for lifted and growing plants respectively.

### SUMMARY

The construction and use of a simple apparatus for recording the swelling of an onion bulb is described. Some specimen records are reproduced in which daily cycles in the rate of swelling are shown. The cause of these fluctuations is briefly discussed and evidence presented that they are mainly effects of temperature. The onset of bulbing may be detected within two days of the first application of the stimulus of long days.

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# A Device for the Study of the Effects of Dissolved Gases upon Tissues or Organisms in Drop Cultures

BY

O. V. S. HEATH

*(Research Institute of Plant Physiology, Imperial College of Science and Technology, London)*

With two Figures in the Text

THE well-known 'Ward's tubes', used for the study of the effects of gases upon hanging-drop cultures, and indeed this culture method in general, are subject to three main disadvantages: (1) the distance of the culture from the microscope condenser is so great that it is impossible to focus the latter even approximately upon the object; (2) the curved lower surface of the hanging drop acts as a lens and still further impairs the quality of the image formed by the objective; (3) it is impossible after setting up the hanging drop to add or remove liquid. The apparatus here described was designed to avoid these disadvantages. It consists of a shallow cell made by cementing four strips, cut from a microscope slide 1 mm. thick, to a thin  $3 \times 1\frac{1}{2}$  in. slide so as to enclose a square as shown in Fig. 1. A gas inlet tube (I) and exit tube (E) of 1-mm. diameter glass capillary are fitted between the strips at opposite corners of the square, and two other capillary tubes (A and B) for introducing and removing liquid are fitted at a third corner. The strips are cemented to the slide with paraffin wax ( $56^{\circ}$  C. m.p.) and the capillaries with sealing-wax. Care must be taken that the capillaries are flush with the strips so that the cover-glass (G) may be down properly with only a thin layer of vaseline. The outer end of each capillary is bent up slightly out of the plane of the slide, and is enlarged by a spindle-shaped coating of sealing-wax to a size suitable for attaching cycle-valve rubber tubing. Bending the capillary tubes, which is of course completed before fitting, presents no difficulty, for in none of them do two bends have to be made in exactly the same plane nor need the angles be exact. A barrier of paraffin wax is melted into position between the two tubes A and B to prevent the liquid of the drop culture from running up into the angle between them, and a ring of paraffin wax is painted on the cover-slip to confine the drop in the centre of the cell. This ring should be of such a size that the two tubes A and B just enter the drop and are almost tangential to it.

In setting up the apparatus the tubes A and B are filled with the liquid by placing a small drop at the inner end of each; a small cap of stoppered valve-rubber tubing used as a teat assists in wetting the interiors of the tubes. A larger drop of the liquid is placed inside the ring on the cover-slip and the

tissue arranged or the organisms added. The cover-slip is then inverted over the cell and bedded down on vaseline. Assuming that enough liquid has been used to make contact with the tubes A and B, more liquid may be added, or some withdrawn, and the drop may be irrigated with vital stains, nutrients, or other reagents as desired. The tubes A and B are left capped (as A, Fig. 1) when not in use; gas is fed in through a length of cycle-valve tubing attached to I and removed via E to a small escape trap.

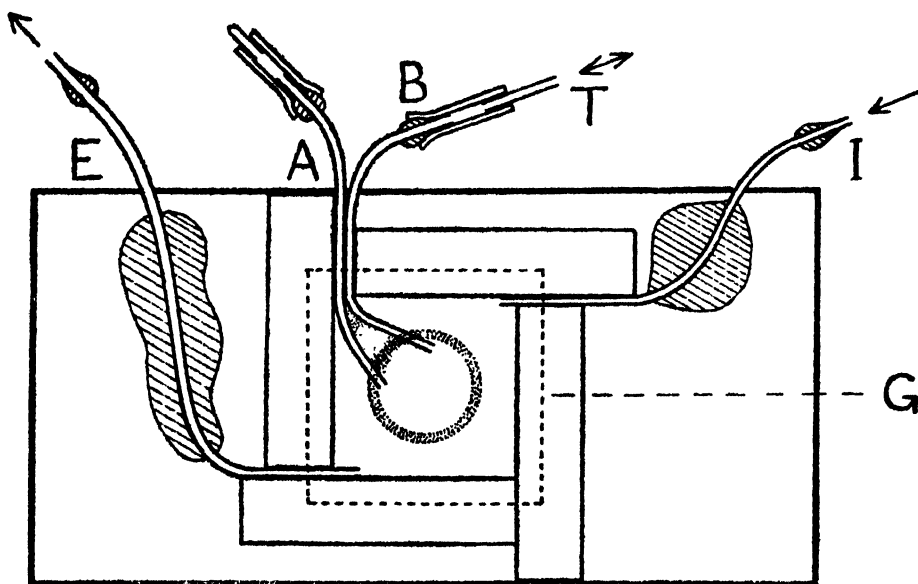


FIG. 1. Cell for liquid drop culture. (For explanation see text.)

Since the drop is only 1 mm. thick and bounded above and below by plane surfaces, it is possible to focus either the top or the bottom of the cell with an ordinary  $\frac{1}{8}$ -in. objective with the condenser in perfect focus at either level. Excellent images can thus be obtained for any level with a  $\frac{1}{6}$ -in. objective, and for the upper surface a  $\frac{1}{2}$ -in. oil-immersion objective may be used.

The chief disadvantage of the apparatus lies in the relatively small surface of the liquid exposed to the gas. This may be largely overcome by the use of a small or even of a U-shaped drop, but the disadvantage is also offset by the possibility of stirring the drop. Occasional stirring may be effected by pinching with forceps the rubber cap closing A or B or continuous agitation and circulation may be produced by means of an automatic pump producing a small oscillation of pressure in either A or B. Owing to the almost tangential position of the tubes such an oscillation produces a somewhat jerky but continuous circulation of the liquid in the drop. Any small oscillating pump would serve, but the type shown in Fig. 2 has the advantage that no mercury or lubricant is employed inside the system and it has proved quite satisfactory. It is adapted from an ordinary mercury relay switch and the diagram is



almost self-explanatory. The principle is similar to that of an electric bell: every time contact is made the magnets *M* attract the armature *N* and so lift the contacts *C* out of the mercury cups; the current is thus switched off and the contacts fall back by gravity into the cups, thus repeating the cycle of events. A rubber washer surrounding and just higher than the projecting portion of the core of one magnet prevents the armature from touching the metal of the cores and makes the pump almost silent in operation. A  $1\mu\text{F}$  condenser across the mercury contacts almost entirely prevents sparking. A piece of brass wire *w* soldered to the lever near the armature presses

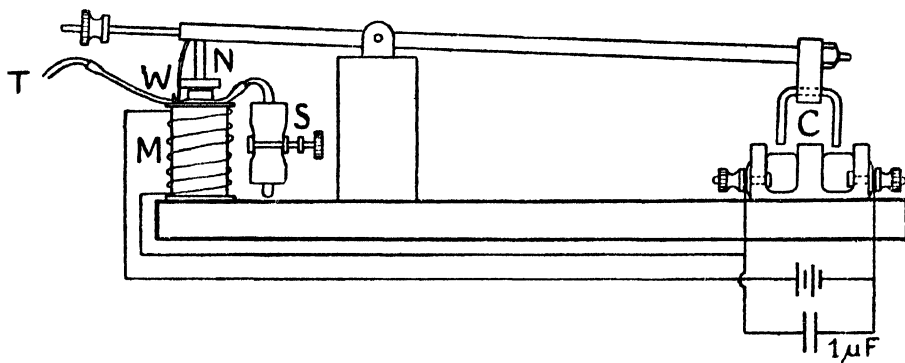


FIG. 2 Pump for automatic stirring (For explanation see text)

intermittently on a short length of cycle-valve tubing and so produces the necessary oscillations of pressure in the glass capillary-tube *T* connected to *B*. At the other end of the system is a stoppered rubber tube and a screw clip *s* by means of which pressure changes due to temperature alterations may be corrected and the oscillating meniscus in *B* adjusted to a convenient position.

Some approximate tests of the efficiency of the automatic stirring were carried out using a drop of distilled water containing a mixed indicator of pH range 8.0 to 4.0. When pure carbon dioxide was passed through the cell, stirring reduced the time necessary to lower the pH to 4.5 throughout the drop to  $\frac{1}{4}$ th of that taken by diffusion alone. In removing carbon dioxide from the drop by passing carbon-dioxide-free air through the cell, the corresponding factor was  $1/4.4$ , but owing to the considerable errors involved in such estimations the difference from the factor for entry of carbon dioxide may be fortuitous. There is little doubt, however, that the tests show a real and large improvement due to the stirring.

The apparatus has been used for investigation of the action of dissolved gases on pieces of leaf tissue and especially on the stomata, but there appears no reason why it should not also be used for the study of fungi, algae, or unicellular organisms. Approximate sterilization can be effected by washing the tubes with alcohol and careful flaming. Even if the wax melts, the strips remain in position unless disturbed. For more rigorous sterilization a harder

cement (e.g. de Khotinsky) could be used throughout, but the advantage of paraffin wax and sealing-wax is that they allow of the whole cell being dismantled for cleaning by heating the under surface of the slide with a small flame.

Since the apparatus not only enables the gas surrounding the drop to be changed but also the fluid of the drop itself, it seems that it should be of use for a considerable range of experiments both in research and in teaching.

# The Distribution of Weight Change in the Young Tomato Plant

## II. Changes in Dry Weight of Separated Organs, and Translocation Rates<sup>1</sup>

BY

D. W. GOODALL

*(The Research Institute of Plant Physiology, Imperial College of Science and Technology, London, S.W. 7, and the Experimental and Research Station, Cheshunt)*

With thirteen Figures in the Text

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### INTRODUCTION

IN the first paper of this series (Goodall, 1945) it has been explained how the present investigation arose as a continuation of the earlier physiological work at Cheshunt (Bolas and Melville, 1933; Bolas et al., 1938). In that paper it was stated that the present research fell into two principal parts—first, the determination throughout the year of the net dry-weight change occurring in each organ of the 8-leaf tomato plant during four periods of the day; and second, the analysis of this net dry-weight change into that part for which translocation to or from the organ was responsible, and the other part representing the net assimilation or respiration of the organ during the period. It is with the second half of the research that the present paper is concerned.

### REVIEW OF LITERATURE

Many workers have made observations on the changes in the rate of assimilation of the leaves during the day; while in some cases a regular curve

<sup>1</sup> The experiments described in this paper formed the basis of a thesis submitted for the degree of Doctor of Philosophy in the University of London in June 1941. Much of the work was performed during the writer's tenure of a Beit Scientific Research Fellowship.

[*Annals of Botany*, N.S. Vol. X, No. 40, October, 1946.]

with a maximum at midday has been found (Curtel, 1890; Kostytschew et al., 1930; Thomas and Hill, 1937; Christopher, 1937), in temperate regions a morning maximum is more common (Kostytschew et al., 1926; Kostytschew and Berg, 1930; Schoder, 1932; Killian, 1933; Bosian, 1933). An afternoon maximum has been observed in *Betula* (Kostytschew et al., 1926) and *Vitis* (Schanderl, 1930) but is unusual. Many workers have observed bimodal assimilation curves, with a depression often about midday, but since in the present work the daylight period was divided into two portions only, these results need not be discussed. It is doubtful to what extent the form of the diurnal curve of assimilation can be related to changes in environmental conditions. Light has been generally considered to be the principal factor (Killian, 1933), but Schoder (1932) found the data of light, temperature, and carbon dioxide concentration together inadequate to explain her curves, and Kostytschew et al. (1928) were of opinion that the variations in assimilation rate through the day had no relation to the environmental conditions.

Observations on the course of respiration during the night are few. Thomas and Hill (1937) with wheat and lucerne, and Christopher (1937) with tomato, found a continuous decrease in the rate of respiration through the night, which the former authors ascribed to the falling temperature.

As regards the diurnal course of translocation, the value of many of the observations is vitiated by the methods used; Godlewski (1873), for instance, found that the disappearance of starch from the cotyledons of *Raphanus* took place more rapidly by day than by night, which he ascribed to the effect of temperature on translocation. Saposchnikoff (1890) found the rate of decrease in carbohydrate content was most rapid in the first hours after sunset. Kostytschew et al. (1926) concluded that in general translocation went on during the night at a similar rate to that during the day. In *Betula* the rate during the day closely followed the rate of assimilation. In *Lappa* the maximum rate occurred in the late morning or in the afternoon; two-thirds of the translocation took place during the light period. In *Phragmites* most of the translocation took place during the afternoon. Schanderl (1930) found that in the vine translocation took place mainly in the late afternoon, to a less extent in the morning, and was negligible at night. Tschesnokov and Bazyrina (1930) found that in *Petasites* also translocation took place only during the day-time, and mainly in the afternoon. In the potato translocation was most active during the afternoon and evening, while in the pea it was greatest at midday and in the early afternoon. In *Tussilago*, on the other hand, a large part of the translocation took place during the early part of the night. In Crafts' (1931) single experiment with *Helianthus* the translocation during the day was over five times as rapid as during the night. Curtis and Herty (1936) made no measurements by day, but from the magnitude of the nocturnal translocation rate concluded that the major part of the translocation in *Phaseolus* took place during the day.

Researches on the effect of external conditions on assimilation and respiration are numerous and need not be reviewed here. There have, however, been

few investigations on the effect of external conditions on translocation. Saposchnikoff (1890) reported that the rate of translocation from the leaf was greater in summer than in winter. On the other hand, Selman (1934) found that the *proportion* of assimilate translocated from the leaf was higher in winter; moreover in summer this figure was inversely related to the light intensity. Curtis (1929) made a number of observations on the effect of temperature on translocation by surrounding the petioles of bean leaves with tubing through which water at the desired temperature was flowing; little effect of temperature was found between 8° and 25° C., though lower temperatures had a considerable retarding effect. In later experiments (Curtis and Herty, 1936) the translocation from leaves with the petioles at 10° C. was found to range between 0.5 and 1.0 per cent. of the leaf dry weight per hour, while at 20° C. it was from 0.9 to 1.5 per cent. per hour. The authors suggested that the low rate of translocation usually found at night was largely due to the low temperature prevailing.

Information on the effect of internal conditions on translocation rate is also scanty. Saposchnikoff (1890) found that the removal of some of the leaves increased the rate of loss of carbohydrate from the remainder—which he took to indicate that translocation rate was affected by the demand for the materials translocated. On the other hand, Tschesnokov and Bazyrina (1930) suggested that the rate of translocation was a function of the amount of surplus assimilate in the leaf. Selman (1934) found that during the summer translocation rate was correlated with assimilation. Phillis and Mason (1939) also found such a correlation under varying conditions of potassium supply.

It has been established (Irving, 1910; Briggs, 1920; Agati, 1937) that the very young leaf does not assimilate, and that the development of assimilatory activity at first lags behind the development of chlorophyll (Singh and Jha, 1939). On the other hand, when two leaves, both mature but of different age, have been compared it has usually been found that the younger one assimilates more actively (Walther, 1927; Gregory and Richards, 1929; Singh and Lal, 1935). Pope (1935), Smirnow et al. (1928), and Gregory and Sen (1937) all report a decrease in respiration rate as the leaf approaches maturity, though the latter two papers both include evidence that there may be an increase after maturity. There is no direct evidence as to the effect of the age of a leaf on the rate of translocation to or from it, though it is evident that in the very early stages the leaf must be dependent upon material translocated to it from the rest of the plant, and it is known that under abnormal conditions the mature leaf, too, can receive translocated material (Mason et al., 1936).

#### METHODS OF EXPERIMENTATION

If investigations dealing only with the mineral elements in the plant are excluded, the methods used for translocation determinations may be grouped into three categories: (i) Respiration is neglected, and the dry-weight of a non-assimilating organ is regarded as representing translocation to or from it.

This has been done with tubers (Denny, 1929), roots (Watson and Baptiste, 1938; Bolas et al., 1938), tree-trunks (Gäumann, 1935), and in particular with darkened leaves. Sachs (1884) and recently Porter (1937) used nocturnal dry-weight losses of leaves as a measure of translocation rates during the day; Müller (1904), Kostytschew et al. (1926), and Guttenberg (1927, 1928) are among those who have regarded the dry-weight losses of leaves darkened during the day as a measure of translocation during the same period from illuminated leaves. Guttenberg's observation that an illuminated leaf of *Quercus* will sometimes lose weight at a greater rate than the leaf simultaneously darkened lends point to the objections often made to this method, as applied to leaves. (ii) Increases or decreases in the quantity or concentration of carbohydrates in the organ are taken as representing translocation to or from it. From the time of Sachs many workers (e.g. Sachs, 1884; Stănescu, 1927; Mason et al., 1936; Palmquist, 1938) have used the appearance or disappearance of starch in a leaf as a qualitative indication of translocation. Grainger (1938) regarded the appearance of reducing sugars in the leaf as an indication of the time of day at which translocation was taking place. Combes and Kohler (1922) and Kusmenko (1938) took diminution in the soluble carbohydrate content of the leaf to represent translocation from it. Davis et al. (1916) and Engard (1939) used changes in the total concentration of carbohydrates as a measure of translocation. But it is known (e.g. Tollenaar, 1925) that many factors may affect the starch-sugar equilibrium, and that carbohydrates may be utilized in cellulose formation and protein synthesis; it is therefore only in special cases, if at all, that changes in the carbohydrates can be regarded as indicative of translocation. (iii) The dry-weight increase of a plant organ and its assimilation or respiration are determined simultaneously, and the difference between the two figures is taken as the translocation. Schanderl (1930) and Tschesnokov and Bazyrina (1930) measured dry-weight change of leaves by Thoday's (1909) modification of Sachs's method, and simultaneously measured the gas exchange of similar leaves. Crafts (1931) used the dry-weight change of a detached leaf as a measure of assimilation or respiration for this purpose, while Curtis and Herty (1936) used that of a leaf with the petiole scalded.

The difference between the dry-weight change of an organ and its assimilation or respiration is evidently the most direct method for measurement of translocation—and even this assumes no change in the relative amount of 'bound' water in the dry matter. But its validity depends upon the identity or the perfect comparability of the leaves or other organs used for the two determinations—a condition which may not be fulfilled if the experimental method used for the assimilation, respiration, or dry-weight change determinations affects the behaviour of the leaf. When a leaf is enclosed in a chamber for assimilation determinations, its assimilation rate is affected by the form of the chamber, and by the rate of flow of air through it (Heinicke and Hoffman, 1935); moreover, the temperature of the leaf in the chamber is likely to be higher than that of one in the open air. It will thus be only by

chance and momentarily that the assimilation of a leaf in a chamber and that of an unenclosed leaf are the same. Therefore, translocation determinations by comparison of assimilation determined by this method and dry-weight change will be unreliable unless the leaves used for the dry-weight determinations are also enclosed in similar leaf chambers. Thomas and Hill (1937) in fact used a very large assimilation chamber covering a plot of ground 6 ft. square, and so were able to carry out the dry-weight change determinations on the same leaves whose assimilation was being measured. Since considerable replication is necessary in dry-weight change determinations, and since it was intended to carry out simultaneous observations on each leaf of the plant separately, such a method was not suited to the present investigation.

The alternative was the use of dry-weight changes of detached organs as estimates of assimilation or respiration for comparison with the dry-weight changes of organs in their normal relations with the rest of the plant. This, however, is also open to considerable objections. Brown and Escombe (1905) found that the carbon dioxide absorption of detached *Helianthus* leaves supplied with water was 30–50 per cent. higher than that of similar leaves on the plant. Bauer (1935) also found that detached leaves supplied with water would sometimes assimilate more actively than those attached to the plant; if they were not supplied with water, the separation from the plant had no consistent effect. Spoechr and McGee (1923) found that the respiration rate of detached leaves fell below that of leaves on the plant after 5 hours. Many writers (Saposchnikoff, 1890; Dastur, 1925; Kostytschew et al., 1926; Kurssanow, 1933; Neubauer, 1939) have held that accumulation of assimilate, which is likely to be more marked in detached than in normal leaves, tends to inhibit further assimilation. There are thus conflicting views as to whether the separation of a leaf from the plant is likely to affect its assimilation, and if so in what direction. It was therefore thought worth while to test the assimilation of detached tomato leaves. This could be done by determining the dry-weight changes of the separated organs by the methods described in the previous paper (Goodall, 1945), and comparing the total of these changes with that for normal plants determined simultaneously by the same methods.

In 3 batches of 10 plants the leaf lengths were measured, and the plants of one batch were divided into their organs, dried, and weighed; in one of the other two batches the leaves, cotyledons, and stem were cut off and placed in sloping trays exposed to the sun with their bases dipping in water, while the roots were washed and left wrapped in a wet cloth in the dark; the third batch of plants was left beside the leaves and stems of the second batch until 7 hours later, when it, too, was divided up, and both batches were dried and weighed. The estimated changes in dry-weight of the parts of the two batches, and the totals, are shown in Table I.

The estimated increase in dry weight of the separated organs is 40 per cent. higher than that of the whole plants; though this difference does not quite

reach the level of significance, it is large enough to demand attention; possibly the increased water-supply, as suggested by Brown and Escombe (1905), had caused the stomata to open more widely. An alternative explanation, however, would be that the separated leaves were all exposed to full sunlight and were not subject to the mutual shading found in the whole plant; Uhl (1937) concluded that the differences in assimilation rate in the various species of *Pinus* that he studied were largely ascribable to differences in the extent of mutual shading.

TABLE I

*Change in Dry Weight of Separated and Attached Organs*

	Whole plants.		Separated organs.	
	Change (%).	S.E. (%).	Change (%).	S.E. (%).
Cotyledons . . . . .	+ 12.5	4.8	+ 8.8	3.3
Leaf 1 . . . . .	+ 7.9	2.2	+ 13.6	2.8
Leaf 2 . . . . .	+ 11.5	6.1	+ 26.3	7.0
Leaf 3 . . . . .	+ 10.9	2.4	+ 18.3	2.8
Leaf 4 . . . . .	+ 9.0	1.6	+ 18.2	3.3
Leaf 5 . . . . .	+ 14.1	2.4	+ 17.6	4.5
Leaf 6 . . . . .	+ 30.8	4.5	+ 18.6	2.7
Leaf 7 . . . . .	+ 35.9	9.4	+ 12.1	5.5
Leaf 8 . . . . .	- 0.9	10.6	- 47.5	2.8
Stem . . . . .	+ 6.4	3.6	+ 1.7	3.7
Root . . . . .	+ 0.5	5.1	- 0.5	4.5
Total . . . . .	+ 9.4	1.3	+ 13.1	1.5

To test the possibility of a shading effect an attempt was made to arrange the leaves in the same relative positions as in the plant. This was accomplished as follows: Holes were drilled in a piece of  $\frac{1}{2}$ -in. curtain rod at about the same levels and angular divergences as the leaves occupy on the stem of the average tomato plant at the 8-leaf stage. In each of these holes was placed a glass tube of such a size that the petiole of a leaf would readily pass into it. In the 'axil' a small cup communicated with the glass tube. Eight such stands were used. Water was supplied from a tube running over the top of these stands. Over each stand the water welled out through a small hole over which ran a number of woollen threads, each leading to one of the cups, by which means the tubes were kept full of water. Such tubes were supplied for leaves 1-5. The water loss from the cotyledons was found to be sufficiently small to make a continuous water-supply unnecessary; these organs were therefore placed in simple closed tubes full of water, but without a side cup. The smaller leaves also did not require a continuous water-supply, and since they had very short petioles they were allowed to rest with their bases in water in glass cups sealed on to the top of the stand, one side of the cup being extended to support the lamina. The whole stand made up in this way supported the leaves in much the same relative positions as in the average plant. The general appearance of the stand with the leaves in position is shown in Fig. 1.



The cut stems, separated from the roots at the level of the cotyledons, were placed vertically on the greenhouse bench, supported by a framework of wire with their lower ends resting in a dish of water. The roots were washed and kept wrapped in a wet cloth in a dark part of the greenhouse.

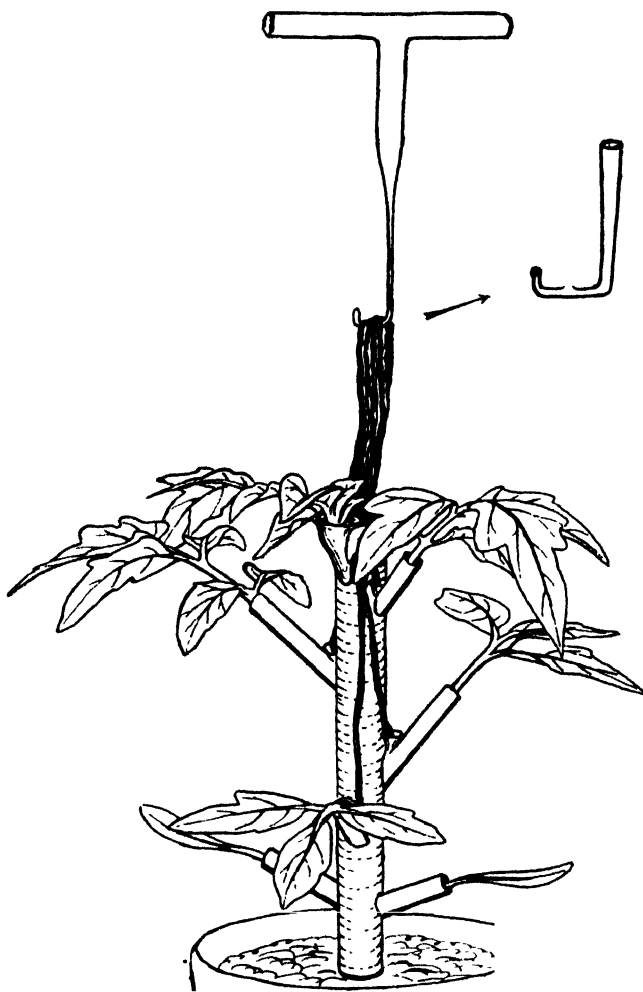


FIG. 1. Assimilation stand with leaves in position.

In the preliminary experiments to compare the total dry-weight change of plant parts arranged in this way with that of a whole plant measured simultaneously, fairly satisfactory agreement was found (Bolas and Goodall, 1937). From these early results, all obtained in the winter, it was concluded that the differences in the behaviour of the separated and attached organs in respect of assimilation could be neglected, though it was realized that this might not also be true of the summer.

*Experimental procedure.*

For determining the dry-weight changes in separated organs, 32 plants were selected in the manner already described (Goodall, 1945) in addition to the 40 used for the observations on dry-weight changes in intact plants, and were divided into 4 batches of 8. The plants of one batch (A) were measured at dawn, with two others (B and C) used for the dry-weight change determinations in the attached organs, and the organs of A were then separated and placed in the stands. The organs of B were placed in the drying oven, and batches A and C remained side by side until noon, when they too were placed in the drying oven. This procedure was repeated for subsequent periods. Thus, over each of the four periods into which the day was divided a single batch of 8 plants served as the common initial sample for both series of dry-weight change determinations—in attached and in separated organs.

*Dry-weight changes: Effect of wilting.*

The possibility of the breaking of the water columns in the vessels of the petiole had to be considered. In winter it was found that the only precaution necessary was to cut with a wet knife and to place the cut end of the petiole immediately in one of the tubes on the stand. In summer, however, the petioles had to be cut under water; even so, in the case of the sample cut at noon the larger leaves sometimes remained severely wilted for an hour or more.

Previous workers had found that the assimilation of wilted leaves is reduced—Nagamatz was quoted by Saposchnikoff (1890) to this effect, and Killian (1933), indeed, found that assimilation ceased in wilted leaves of *Narcissus*. Thoday (1910) found that in separated leaves of *Helianthus* flaccidity diminished assimilation; and Mitchell (1936) reported that in *Cineraria* slight wilting might reduce assimilation by as much as 30–40 per cent. It was accordingly thought necessary to test whether the wilting observed in these experiments was likely to affect the results.

A record was kept of those leaves that had wilted, and their dry-weight increase and that of the leaves of the same batch that had remained turgid were calculated separately. It was found that wilting was in fact associated with a substantially lower net gain in dry weight, as is shown in Table II, the figures of which are drawn from experiments at all times of the year

TABLE II

*Difference in Net Assimilation between Wilted and Turgid Leaves  
during Half-day (as percentage of initial weight)*

Degree of wilting.	No. of leaves.	Difference.	S.E.
Slight . . . .	126	—1·64	1·25
Moderate . . . .	65	—6·27	1·51
Severe. . . . .	35	—9·09	2·09

Therefore, all leaves recorded as wilted were omitted from the calculation. This involved the elimination of the entire data for the older leaves of the afternoon samples on five dates during the summer months.

*Dry-weight change: Effect of separation.*

From the remaining unwilted and apparently normal separated organs the changes in dry weight were calculated in the way described in the previous paper and the total of these changes was compared with that for the organs of the intact plant. This process was carried out for each experiment in the series of 26 experiments, with the results shown in Table III.

TABLE III  
*Difference of Percentage Change in Total Dry Weight of Separated  
Organs from those of Intact Plants*

Date of experiment	Dawn--nudday.		Midday--dusk.		Dusk midnight		Midnight dawn.	
	S.E.		S.E.		S.E.		S.E.	
Dec. 6, 1936	- 2.1	4.2	1.3	4.1	0.9	4.2	+ 11.7	4.1
Dec. 29, 1936	2.1	5.1	1.5	5.0	2.8	5.0	+ 8.4	4.9
Jan. 11, 1937	0.1	5.5	- 1.8	5.6	+ 4.5	5.5	3.0	5.5
Jan. 18, 1937	+ 0.2	4.1	5.7	4.2	+ 3.0	4.1	0.9	4.1
Feb. 4, 1937	+ 1.0	4.3	2.7	4.4	+ 8.2	4.4	+ 7.0	4.4
Feb. 15, 1937	0.9	4.1	3.0	3.9	+ 0.9	4.1	+ 3.6	4.3
Feb. 23, 1937	0.5	4.5	+ 9.3	4.4	+ 1.0	4.4	+ 6.7	4.5
Mar. 8, 1937	+ 4.1	4.4	5.8	3.8	+ 2.3	3.8	- 7.7	4.2
Mar. 23, 1937	3.1	3.3	- 3.1	3.1	+ 1.0	2.9	+ 0.2	3.0
Apr. 5, 1937	+ 4.7	4.6	—	—	- 3.1	4.7	+ 0.7	4.7
Apr. 12, 1937	6.2	5.1	- 2.2	5.2	+ 5.5	5.2	+ 2.8	5.3
Apr. 28, 1937	+ 0.3	3.3	1.1	3.3	+ 0.1	3.3	+ 1.1	3.3
May 6, 1937	+ 5.0	2.9	—	—	+ 0.6	2.7	- 2.1	2.7
May 24, 1937	- 0.2	3.5	—	—	- 1.1	3.6	- 2.7	3.4
June 2, 1937	5.3	3.5	2.0	3.6	1.3	3.5	- 3.7	3.5
July 7, 1937	4.3	3.1	+ 2.7	3.1	0.1	3.1	- 7.2	3.2
Aug. 17, 1937	1.1	5.3	—	—	+ 2.7	5.4	- 1.3	5.4
Aug. 25, 1937	5.3	2.9	—	—	—	—	—	—
Sept. 2, 1937	- 7.7	4.4	6.3	4.3	+ 0.5	4.4	- 7.7	4.4
Sept. 10, 1937	- 3.4	4.8	- 9.4	4.8	3.0	4.8	0.0	4.8
Sept. 27, 1937	- 0.2	4.0	5.7	4.0	- 1.4	4.1	- 1.3	4.0
Oct. 11, 1937	+ 1.0	4.0	- 0.2	4.1	+ 2.9	4.0	- 7.0	4.0
Oct. 21, 1937	—	—	- 5.1	2.9	+ 1.1	2.9	+ 1.1	2.9
Nov. 11, 1937	- 2.0	5.1	+ 0.4	5.2	- 7.8	5.2	- 5.3	5.2
Nov. 25, 1937	- 0.5	3.8	- 0.5	3.9	- 7.9	3.9	- 1.3	3.9
Dec. 7, 1937	- 4.6	3.9	+ 4.3	3.8	+ 1.3	3.9	+ 2.9	3.9
Mean (winter)	- 0.5	1.3	1.4	1.2	+ 0.3	1.2	+ 1.1	1.2
Mean (summer)	- 2.2	1.2	- 3.1	1.6	+ 0.6	1.3	- 1.8	1.3
Mean (all expts.)	- 1.3	0.9	- 1.9	0.9	+ 0.4	0.9	- 0.2	0.9

The figures given for the standard error are maximum values (Goodall, 1945).

From this table it will be seen that the conclusions drawn from the early experiments in winter were unjustified, and that during the daytime the separated organs may gain in dry weight at a lower rate than the intact plants. This is in contrast with the results of Brown and Escombe (1905)

and of Bauer (1935), who found that detached leaves supplied with water assimilated more than those remaining on the plant. Table II shows also that the difference is more marked in the afternoon than in the morning, especially in view of the lower rate of gain in the intact plant after midday. The accumulation of assimilates in the detached leaves may have been responsible for the lowered assimilation (Saposchnikoff, 1890; Kurssanow, 1933)—a point of view supported by the fact that the effect is more marked after midday. Another possibility is that these leaves, though appearing normal, were in a condition of incipient wilting through inadequate water-supply; the difference of their dry-weight change from that of the intact plant is of the same order as that caused by slight wilting (see Table II), and the results of Dastur (1925) and Melville (1937) indicate that assimilation may be affected by leaf water-content. Yet a third possibility is that the absence of a mineral supply to these leaves was responsible.

During the first half of the night the differences between the separated and the intact batches are not significant, though they suggest a less rapid respiration in the former. In summer during the second part of the night there is a considerable deviation in the opposite direction—that is, the detached organs gain less than the intact plant; this difference, however, does not reach significance.

#### EXPERIMENTAL RESULTS

##### *I. Dry-weight changes of separated organs.*

Table IV gives the mean values for dry-weight changes taking place at different times of day in the separated organs.

From the table it may be seen that the roots were respiring and losing in dry weight in most periods, the average rate being about 0.5 per cent. per hour. Unfortunately the errors in dry-weight change estimation for this organ were so large that the differences between the average rates for different periods are in most cases not significant. The only exception is that the loss of dry weight during the summer evening is significantly greater than during the winter evening, although the mean temperature in summer was slightly lower. It is possible that a greater concentration of labile carbohydrates may have been responsible for this difference; it cannot be ascribed to a greater rate of translocation from the stem, as Harris (1929) has done with similar results on the respiration of apple roots, since translocation here has not been taking place.

The stem was also almost always losing in weight, in spite of its chlorophyll. At all times of day the average rate of loss was greater in summer than in winter. As in the root, the greatest rates of loss occurred in the summer evenings, but the average net loss of weight of the stem was throughout less than that of the root. This may in part be due to the small amount of assimilation taking place in the stem; the differences during the night periods are not significant, though they are in the same direction.

TABLE IV

*Change in Dry Weight (per cent. per hour) of Separated Organs of the Tomato Plant throughout the Twenty-four Hours. Mean Values are given for Winter and Summer, and for the Year as a whole<sup>1</sup>*

		Dawn-midday.		Midday-dusk.		Dusk-midnight.		Midnight-dawn.		
		S.E.		S.E.		S.E.		S.E.		
Root										
Winter	.	.	- 0 74	0 38	- 0 39	0 37	+ 0 21	0 28	- 0 17	0 29
Summer	.	.	- 0 88	0 26	0 51	0 26	1 26	0 57	0 24	0 54
General mean	.	.	- 0 81	0 23	- 0 44	0 23	- 0 44	0 32	- 0 20	0 29
Stem										
Winter	.	.	- 0 01	0 28	+ 0 08	0 27	+ 0 18	0 20	- 0 12	0 21
Summer	.	.	- 0 44	0 20	- 0 01	0 20	- 0 91	0 39	- 0 18	0 36
General mean	.	.	0 22	0 17	+ 0 04	0 17	0 30	0 20	0 15	0 20
Cotyledons										
Winter	.	.	+ 1 10	0 54	0 26	0 47	+ 0 44	0 38	- 0 10	0 36
Summer	.	.	+ 0 74	0 25	+ 1 09	0 31	- 0 22	0 45	+ 0 36	0 43
General mean	.	.	+ 0 89	0 27	+ 0 30	0 31	+ 0 11	0 30	+ 0 14	0 29
Leaf 1										
Winter	.	.	+ 1 01	0 25	+ 0 75	0 24	0 10	0 19	+ 0 03	0 19
Summer	.	.	+ 2 02	0 21	+ 0 92	0 28	1 06	0 43	+ 0 14	0 41
General mean	.	.	+ 1 50	0 16	+ 0 81	0 19	- 0 52	0 22	+ 0 08	0 21
Leaf 2										
Winter	.	.	+ 1 10	0 29	+ 1 03	0 26	+ 0 03	0 21	+ 0 07	0 21
Summer	.	.	+ 2 16	0 21	+ 0 90	0 28	- 1 13	0 40	0 29	0 38
General mean	.	.	+ 1 61	0 18	+ 0 90	0 20	- 0 48	0 21	- 0 09	0 21
Leaf 3										
Winter	.	.	+ 1 65	0 27	+ 1 56	0 25	0 17	0 19	0 04	0 20
Summer	.	.	+ 2 30	0 16	+ 1 23	0 22	0 45	0 34	+ 0 35	0 32
General mean	.	.	+ 1 96	0 16	+ 1 46	0 19	0 29	0 19	+ 0 18	0 18
Leaf 4										
Winter	.	.	+ 1 93	0 38	+ 1 12	0 25	- 0 07	0 19	0 19	0 19
Summer	.	.	+ 1 98	0 18	+ 0 93	0 21	0 56	0 32	+ 0 28	0 30
General mean	.	.	+ 1 95	0 17	+ 1 06	0 19	- 0 23	0 18	+ 0 02	0 17
Leaf 5										
Winter	.	.	+ 0 83	0 29	+ 1 34	0 27	+ 0 01	0 19	- 0 30	0 20
Summer	.	.	+ 1 44	0 19	+ 1 06	0 22	0 43	0 36	0 17	0 34
General mean	.	.	+ 1 12	0 18	+ 1 25	0 20	- 0 18	0 19	- 0 24	0 19
Leaf 6										
Winter	.	.	+ 0 30	0 34	+ 0 80	0 32	- 0 51	0 23	- 0 42	0 24
Summer	.	.	+ 0 74	0 19	+ 0 52	0 25	- 0 15	0 42	+ 0 62	0 39
General mean	.	.	+ 0 51	0 20	+ 0 70	0 23	- 0 35	0 22	+ 0 04	0 22
Leaf 7										
Winter	.	.	+ 1 34	0 55	+ 0 54	0 51	- 0 46	0 37	+ 0 09	0 38
Summer	.	.	0 01	0 29	+ 0 06	0 29	- 2 14	0 61	1 24	0 56
General mean	.	.	+ 0 66	0 31	+ 0 31	0 30	- 1 23	0 35	0 52	0 33
Leaf 8										
Winter	.	.	- 1 44	1 00	+ 0 77	0 98	- 0 27	0 75	- 0 52	0 70
Summer	.	.	+ 0 44	0 44	+ 0 60	0 45	- 4 10	0 97	- 0 58	0 88
General mean	.	.	- 0 46	0 53	+ 0 69	0 53	- 1 82	0 60	- 0 55	0 56

<sup>1</sup> Data of each of the 26 experiments are given separately in the original thesis.

The remaining organs—the cotyledons and the foliage leaves of different ages—may conveniently be discussed together. In general, as might be expected, they gained in dry weight during the day and lost during the night; this rule is without significant exceptions among the summer and winter means. The rate of gain before midday was in most of the leaves substantially greater than during the afternoon; as already suggested (p. 314), this might have been due to restriction of assimilation by the accumulation of assimilates; or alternatively, to the different water relations of leaves separated from the plant at dawn and at noon. In addition, the lower light intensity during the afternoon doubtless played a part. The loss of dry matter during the night was much more marked before than after midnight—which may well be because the concentration of respiratory substrate was greater in the former period; this result is parallel with those cited on p. 306. As between summer and winter, before midday the increase in dry weight was more rapid in summer than in winter in all leaves except the seventh and the cotyledons, while in the afternoon the reverse was true, with the exception of the cotyledons and the first leaf. In the evening, loss of dry weight was in general more rapid in summer, but after midnight there is less regularity.

The rates of change in these organs considered as a series of increasing age are the main interest of these figures. In Fig. 2 are plotted the mean rates of dry-weight increase (per cent. per hour) for the whole day-time—that is, from dawn to dusk. In both summer and winter there is a marked peak at the third leaf. The falling-off in assimilation towards the older leaves is much more noticeable in the winter than in the summer, which is in agreement with the more rapid senescence of the leaves at that time. It should be noted that the third leaf, both in winter and summer, is still by no means mature, and if the plant does not become ‘pot-bound’ may reach a length more than double that at the time of sampling. At maturity, then, the assimilatory activity of the leaf expressed on a dry-weight basis has already passed its maximal value.

Fig. 3 shows in similar fashion the losses during the night as a whole. Here the trend is less pronounced, but the more rapid loss of weight by the younger than by the older leaves is clear. There is also a suggestion of a more rapid loss of weight by the older leaves from the second downward than in the intermediate ones. This is particularly marked in the summer evening.

In Table IV the high rate of loss of dry weight by the eighth leaf during the summer evening is particularly to be noted. The mean rate of loss during this period is 4 per cent. per hour—an unprecedentedly high figure; it may, however, be compared with Gäumann’s (1935) observation that during foliation of the beech half of the material passing into the young leaves was used in respiration.

Up to this point discussion of the changes in isolated leaves has been on a percentage basis. Assimilation rates, however, are more usually and more logically expressed on an area basis—for the absorption of light and of

carbon dioxide by a leaf (the two most usual 'limiting factors') both depend more on its area than on its dry weight. Accordingly, the mean rates for the whole day-time represented in Fig. 2 have been converted to an area basis,

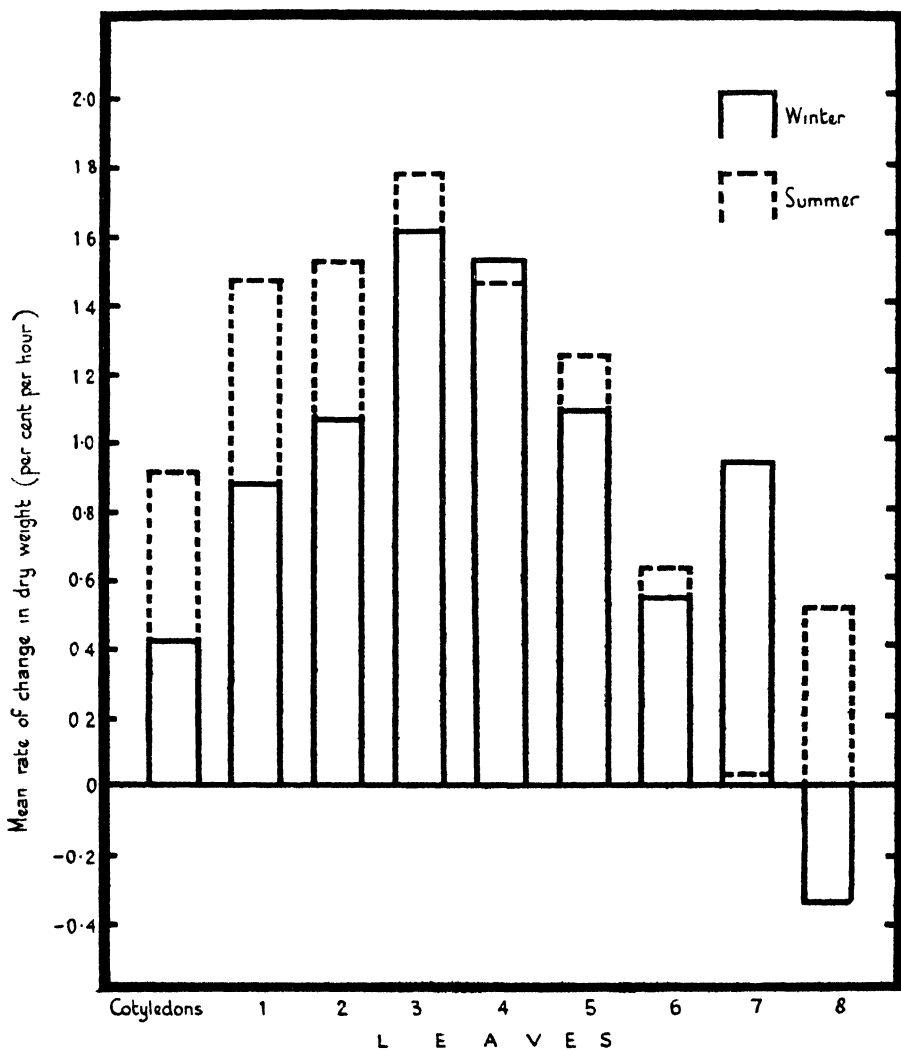


FIG. 2. Mean rate of change in dry weight of separated leaves during day-time.

using the area/dry-weight ratios for leaves of different ages found in the average summer and winter plants; the dry-weight increase, moreover, has been expressed as carbon-dioxide absorption, on the assumption that the assimilate has the formula  $(\text{CH}_2\text{O})_x$ . The resulting values are presented in Fig. 4. Since the weight per unit area increases with age, this has reduced the apparent differences among the older leaves, so that the peak at the third leaf

is not so marked on this basis, whereas the difference between older and younger leaves is greater than on a dry-matter basis. Thus, in so far as one may use data regarding leaves of different ages borne at different levels on the

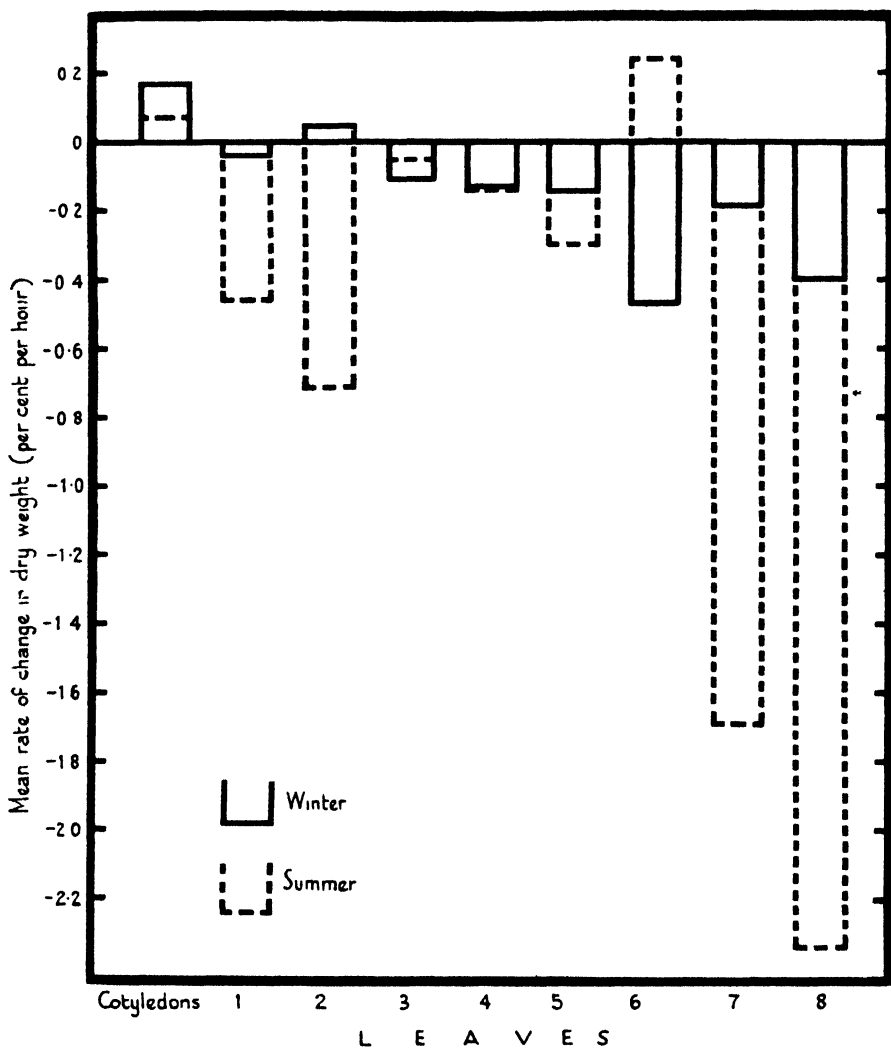


FIG. 3. Mean rate of change in dry weight of separated leaves during night-time.

same plant as a basis for deductions regarding the changes in the course of development of a leaf, during the period of most rapid expansion of the leaf there is also a great increase in the assimilatory activity expressed on an area basis; when the leaf has reached about half its final length this increase ceases, and assimilation per unit area remains fairly constant until senescence sets in.



The assimilation rates shown in the figure are fairly low. The highest is 7.6 mg. CO<sub>2</sub>/dm.<sup>2</sup>/hr., whereas in the literature 20 is a common figure, and values over 60 are not unknown (e.g. Kostytschew and Kardo-Sysoiewa, 1930; Blagowestschenski, 1935); Lundegårdh's (1928) maximum for the

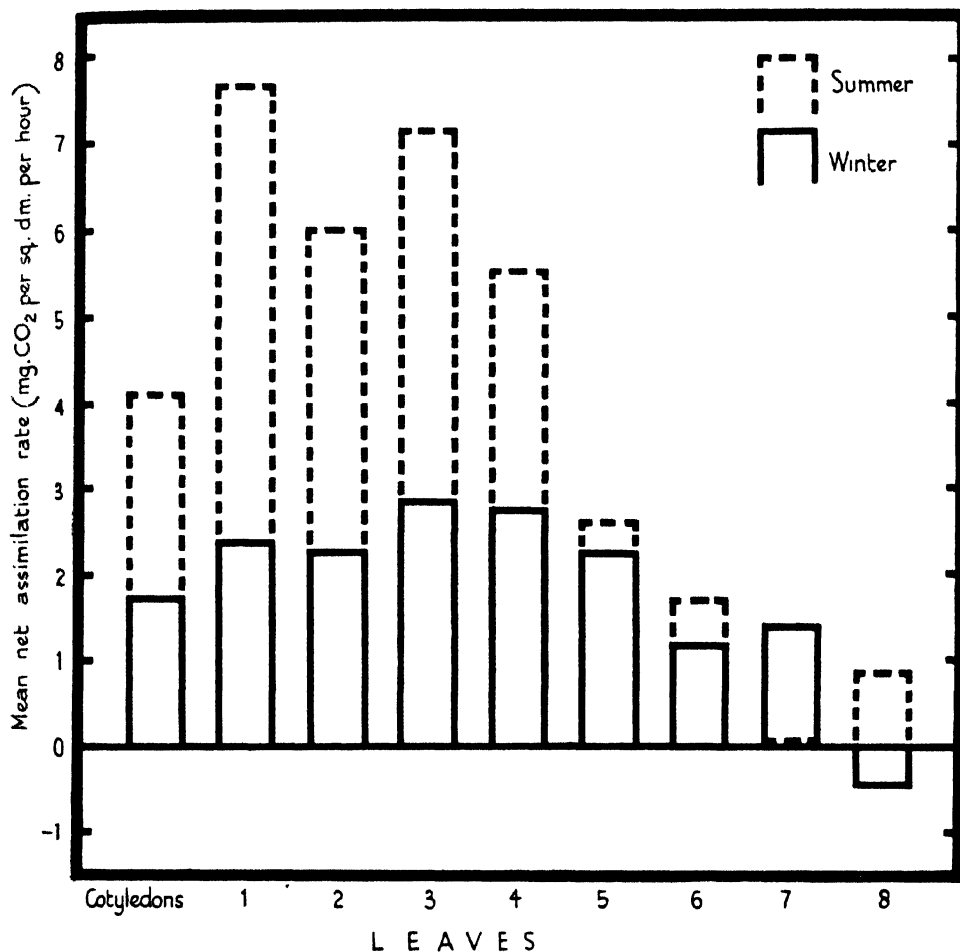


FIG. 4. Mean net assimilation rate of separated leaves during day-time.

tomato was 16.8. It must be remembered, though, that the figure of 7.6 averages the whole period of daylight during 7 days spaced through the 6 months of summer, and so can hardly be compared with values determined over a period of a few minutes or even an hour. The highest individual value for the larger leaves in these experiments is 3.59 per cent. per hour (Aug. 25, 1937, dawn to midday, leaf 1), which represents approximately 18.6 mg. CO<sub>2</sub>/dm.<sup>2</sup>/hr. This is comparable with the maximal figures obtained in many investigations on assimilation under natural conditions.

## II. *Estimated translocation rates.*

Had the total changes in dry weight of the separated organs been the same as those of the intact plant, the rates of translocation to and from the various organs could have been derived from the differences between the figures for dry-weight change of the organs of the intact plant (Goodall, 1945) on the one hand and those of which the means are given in Table IV on the other. Since the total dry-weight changes in the two series differed this could not be done so simply. An approximate estimate of the translocation could, however, be obtained on the assumption that the difference between the total change of the intact plant and that of the separated organs was distributed among the different organs in proportion to their weight. This assumption biases the translocation figures in favour of the initial proportions of the plant, whereas, in fact, translocation is the principal means whereby the proportions of the plant change; as a result of this assumption translocation rates tend to be underestimated.

The procedure by which the calculation was made was as follows: For any one period and experiment the logarithm of the mean final weight of the intact plant was subtracted from the corresponding figure for the total of the separated organs. For the organ in question the logarithm of the final weight in the separated series was subtracted from that in the intact series. These two differences were then added, their sum divided by the duration of the period, and the result expressed as a percentage rate per hour.

In Table V the summer, winter, and general means of these values are given. For the individual values no estimates of error were available on account of the arbitrary nature of the assumption on which the calculations were based. For the means of different periods and different organs, however, some kind of estimate of error can be obtained from the differences between the experiments performed on different dates. These error estimates are included in the table. Even the results that prove to be significant when tested on the basis of these error estimates, however, must be accepted with caution, and it is not claimed that these figures supply anything more than indications of the course which translocation takes in the young tomato plant.

TABLE V

*Estimated Rates of Change in Dry Weight (per cent. per hour) by Translocation by the Various Organs of the Tomato Plant throughout the Twenty-four Hours. Mean Values are given for Winter and Summer, and for the Year as a whole<sup>1</sup>*

		Dawn-midday.		Midday-dusk.		Dusk-midnight.		Midnight-dawn.	
		S.E.		S.E.		S.E.		S.E.	
Root									
Winter	.	+0.59	0.44	+0.12	0.24	+0.64	0.22	-0.06	0.25
Summer	.	+1.68	0.29	+1.38	0.33	+0.56	0.40	-0.10	0.42
General mean	.	+1.11	0.29	+0.70	0.23	+0.61	0.20	-0.08	0.23

<sup>1</sup> Data of each of the 26 experiments are given separately in the original thesis.

TABLE V—(cont.)

		Dawn-midday.		Midday-dusk.		Dusk-midnight.		Midnight-dawn.	
		S.E.		S.E.		S.E.		S.E.	
Stem									
Winter	.	.	+0.36 0.29	.	+0.80 0.21	.	+0.23 0.11	.	+0.16 0.25
Summer	.	.	+1.19 0.13	.	+1.64 0.18	.	+0.73 0.29	.	+0.86 0.20
General mean	.	.	+0.73 0.18	.	+1.25 0.14	.	+0.45 0.14	.	+0.46 0.18
Cotyledons									
Winter	.	.	-0.37 0.51	.	-0.55 0.33	.	-0.90 0.48	.	+0.24 0.55
Summer	.	.	-0.78 0.30	.	-0.70 0.41	.	-0.94 0.22	.	-0.10 0.40
General mean	.	.	-0.62 0.28	.	-0.61 0.25	.	-0.92 0.26	.	+0.05 0.32
Leaf 1									
Winter	.	.	+0.08 0.27	.	0.47 0.13	.	+0.11 0.17	.	-0.20 0.18
Summer	.	.	1.13 0.22	.	-0.67 0.30	.	-0.03 0.26	.	+0.16 0.27
General mean	.	.	-0.50 0.22	.	-0.54 0.13	.	+0.05 0.15	.	-0.04 0.16
Leaf 2									
Winter	.	.	-0.12 0.27	.	-0.78 0.20	.	-0.41 0.16	.	-0.16 0.17
Summer	.	.	-1.19 0.15	.	-0.09 0.35	.	+0.01 0.32	.	-0.16 0.26
General mean	.	.	-0.64 0.18	.	-0.55 0.19	.	-0.22 0.17	.	-0.16 0.15
Leaf 3									
Winter	.	.	-0.52 0.27	.	-0.26 0.22	.	-0.49 0.28	.	-0.29 0.22
Summer	.	.	-0.69 0.18	.	-0.37 0.28	.	-0.75 0.19	.	-0.23 0.26
General mean	.	.	-0.60 0.15	.	-0.29 0.17	.	-0.60 0.17	.	-0.27 0.16
Leaf 4									
Winter	.	.	-0.52 0.17	.	-0.31 0.17	.	-0.18 0.15	.	-0.14 0.16
Summer	.	.	-0.02 0.24	.	+0.15 0.23	.	-0.28 0.30	.	-0.19 0.34
General mean	.	.	-0.28 0.15	.	-0.17 0.14	.	-0.22 0.15	.	-0.17 0.17
Leaf 5									
Winter	.	.	+0.27 0.29	.	+0.53 0.25	.	+0.17 0.12	.	+0.22 0.12
Summer	.	.	+0.59 0.27	.	+1.10 0.25	.	+0.18 0.25	.	+0.64 0.32
General mean	.	.	+0.43 0.18	.	+0.72 0.19	.	+0.18 0.13	.	+0.40 0.16
Leaf 6									
Winter	.	.	+1.03 0.20	.	+1.13 0.29	.	+1.07 0.21	.	+1.09 0.27
Summer	.	.	+1.48 0.29	.	+1.23 0.16	.	+0.36 0.39	.	+0.91 0.47
General mean	.	.	+1.25 0.18	.	+1.15 0.20	.	+0.76 0.22	.	+1.01 0.25
Leaf 7									
Winter	.	.	+1.71 0.38	.	+2.61 0.42	.	+1.19 0.49	.	+1.13 0.40
Summer	.	.	+2.55 0.55	.	+2.71 0.29	.	+1.39 0.33	.	+1.59 0.40
General mean	.	.	+2.14 0.35	.	+2.66 0.24	.	+1.28 0.30	.	+1.35 0.28
Leaf 8									
Winter	.	.	+2.10 0.72	.	+1.41 0.78	.	+2.31 0.80	.	+1.25 0.94
Summer	.	.	+1.73 0.75	.	+2.84 0.52	.	+1.33 1.20	.	+1.02 0.87
General mean	.	.	+1.91 0.51	.	+2.07 0.50	.	+1.84 0.74	.	+1.14 0.63

One striking point which emerges from the results is that translocation was, in all organs, more rapid during the day than during the night; this agrees with most of the previous work on the subject (see pp. 306-7). On average, the rate during the night was about half that during the day. There is a suggestion

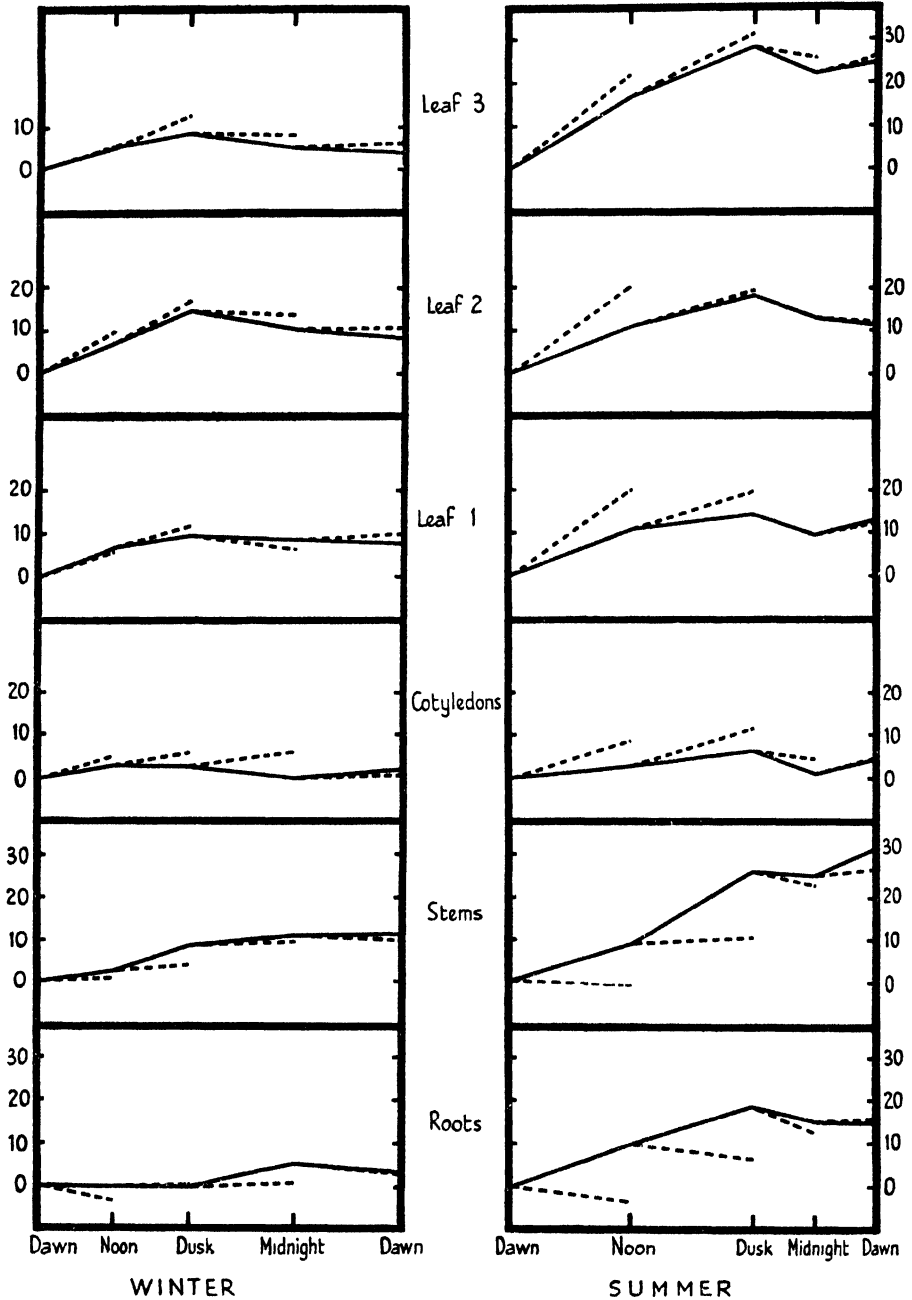


FIG. 5A. Dry-weight change (per cent. of initial weight) of various organs throughout the day; roots, stems, cotyledons, and leaves 1-3. The full line represents the change in the attached organ, the broken line the corrected change in the separated organ.

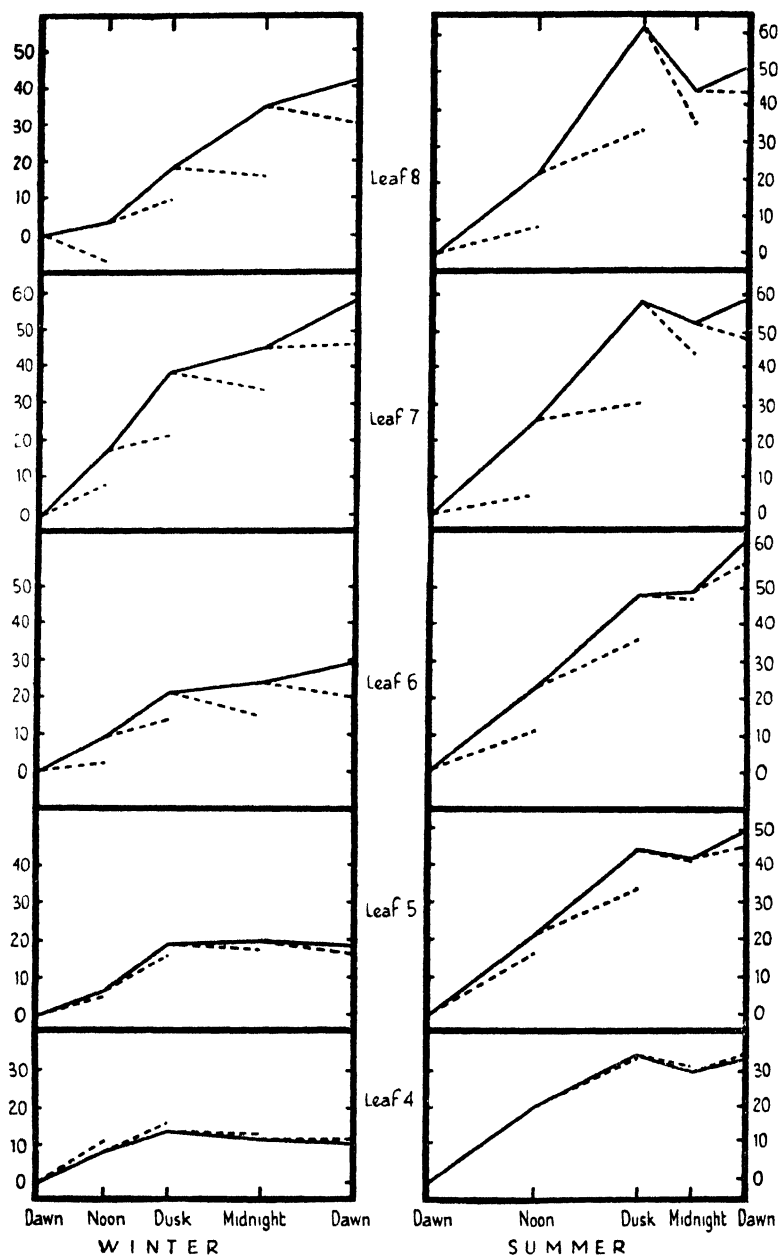


FIG. 5B. Dry-weight change (per cent. of initial weight) throughout the day; leaves 4-8. Further description as in Fig. 5A.

that, in the case of the stems and young leaves at any rate, translocation was more active during the afternoon than before midday. During the night translocation to these organs continued at a fairly constant rate; translocation to the roots, however, had virtually ceased by midnight, and the same is true of translocation from the cotyledons.

Throughout the day, summer and winter, the older leaves—that is, the cotyledons to the fourth leaf inclusive—were suffering a net loss of material by translocation, while the other organs—stem, root, and the younger leaves from the fifth upwards—were always gaining. Even the fifth leaf, with a length of about 7 cm., was by no means self-supporting, since it was gaining by translocation an average of 0.45 per cent. per hour—that is, 11 per cent. per day, or one-third of its total increase. The fourth leaf in summer on balance lost very little by translocation; in winter the loss was considerably greater. This is in accord with the more rapid senescence of the leaves in winter.

TABLE VI

*Mean Net Dry-weight Changes (per cent.) in the Different Organs during Twenty-four Hours*

	Winter.			Summer.		
	Dry-wt. change.	By assimilation or respiration.	By translocation.	Dry-wt. change.	By assimilation or respiration.	By translocation.
Roots	+3.0	-4.8	+7.8	+15.8	-12.4	+28.2
Stems	+10.8	+1.6	+9.2	+31.0	-0.6	+31.6
Cotyledons	+2.2	+11.7	-9.5	+4.5	+20.4	-15.9
Leaf 1	+7.8	+10.5	-2.7	+13.0	+26.7	-13.7
Leaf 2	+7.4	+17.0	-9.6	+13.0	+23.7	-10.7
Leaf 3	+4.3	+13.6	-9.3	+25.3	+39.0	-13.7
Leaf 4	+8.8	+16.3	-7.5	+33.8	+35.0	-1.2
Leaf 5	+19.0	+11.1	+7.9	+49.7	+29.2	+20.5
Leaf 6	+29.2	-2.1	+31.3	+62.3	+30.0	+32.3
Leaf 7	+57.9	+9.4	+48.5	+57.9	8.7	+66.6
Leaf 8	+41.7	-8.0	+49.7	+50.2	-6.7	+56.9

The variations in nocturnal translocation with season—that is, the differences between the summer and winter means—are not noteworthy except in the stem, where translocation at night was more than twice as active in summer as in winter. During the day-time this difference was more general, being very significant in the root and stem, and in the same direction though not significant in the younger leaves. It must be noted that these results refer to the rate of translocation per hour, and not to the total amount translocated in the day. Since translocation was more rapid during the day than the night, and since the day is so much longer in summer than in winter, the total amounts translocated during the 24 hours in winter and summer would show even greater differences than do the rates of translocation.

In Fig. 5 the mean translocation rates for summer and winter for the various organs are compared with the rates of dry-weight increase. In each graph the

full line represents the changes of the organs forming part of the intact plant, while the dotted lines represent the adjusted changes of the separated organs. These figures are derived from the winter and summer means for the rates of dry-weight change (Goodall, 1945) and translocation rates (Table V), together with the mean durations of the four periods into which the day is divided; all the changes are expressed as percentages of the weight of the

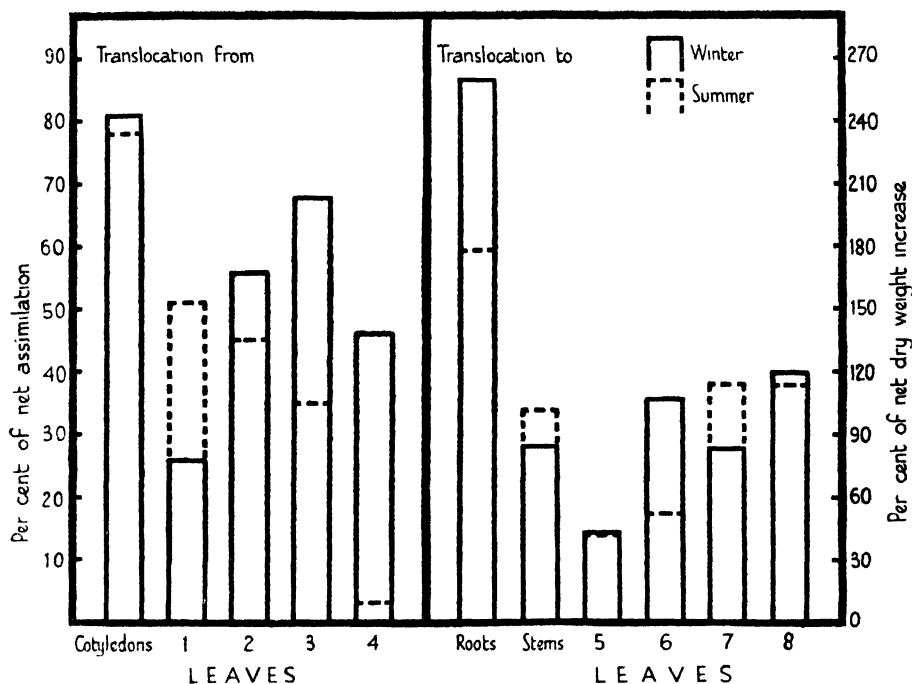


FIG. 6. Translocation over the 24 hours expressed, for the older leaves, as a percentage of the organ's net assimilation and, for the other organs, as a percentage of the net dry-weight increase.

initial dawn sample. In Table VI the same figures are expressed as totals for the 24 hours.

From Table VI and Fig. 5 it will be seen that about half of the material translocated to the root was used in respiration; Harris's (1929) figure for apple roots was from two-thirds to three-quarters. The stem contains chlorophyll, and seems to be able to supply enough assimilate for its own respiration, the material translocated to it thus being all available for the building of new tissue. The smaller leaves did not assimilate enough to compensate for their high respiration rates at night, but the proportion of the material translocated to them used for respiration was not large; by contrast, Gäumann (1935) found that young beech leaves respired half of the material translocated to them. Regarding the reverse side of the picture, of the organs that were supplying the rest of the plant with material the cotyledons, both in winter and summer, were parting with more than three-quarters of their net

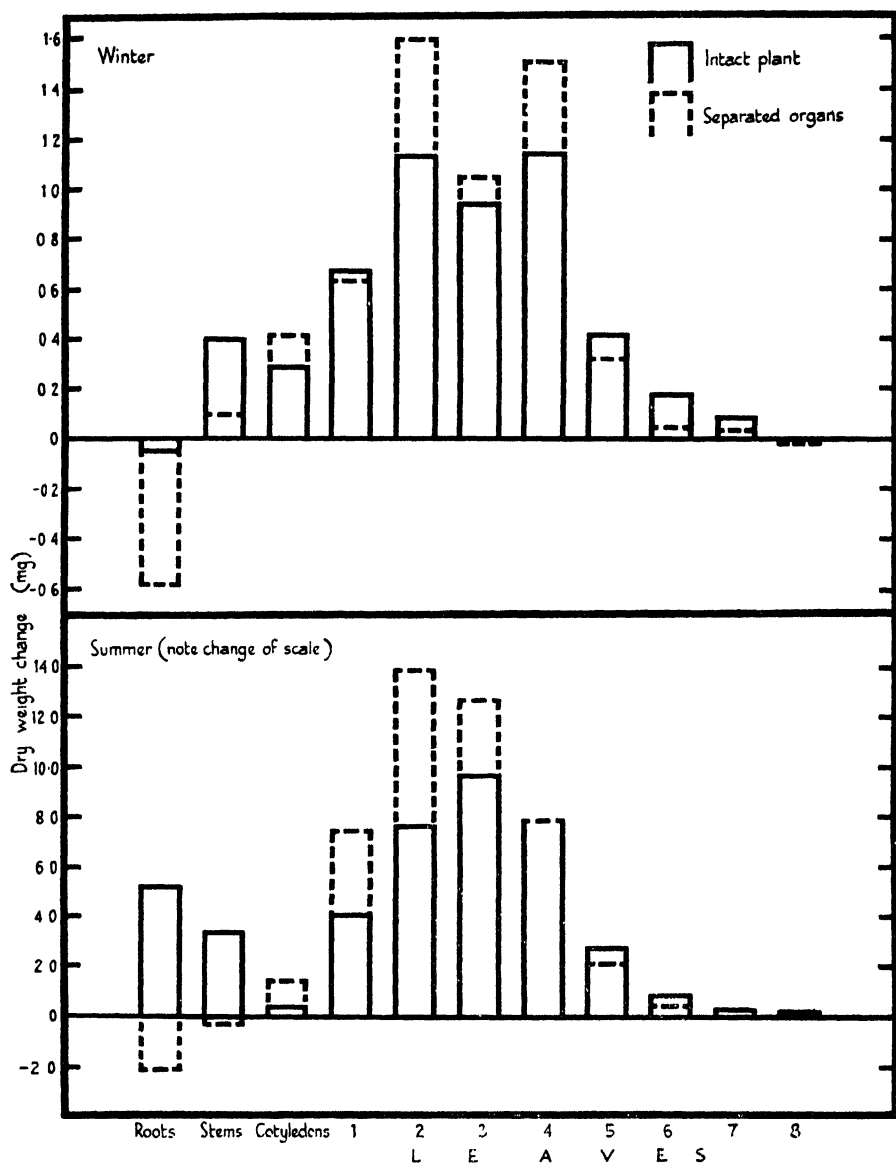


FIG. 7. Absolute changes in dry weight of intact plants and separated organs (corrected) during the morning.

assimilate. In the winter data the proportion of their assimilate given up by the leaves does not decrease very rapidly in passing from the older to the younger ones—even the fourth leaf lost 40 per cent. of its assimilate by translocation; in summer the gradient was much steeper, so that the fourth leaf lost only 3 per cent. of its net assimilate by translocation. In Fig. 6 translocation



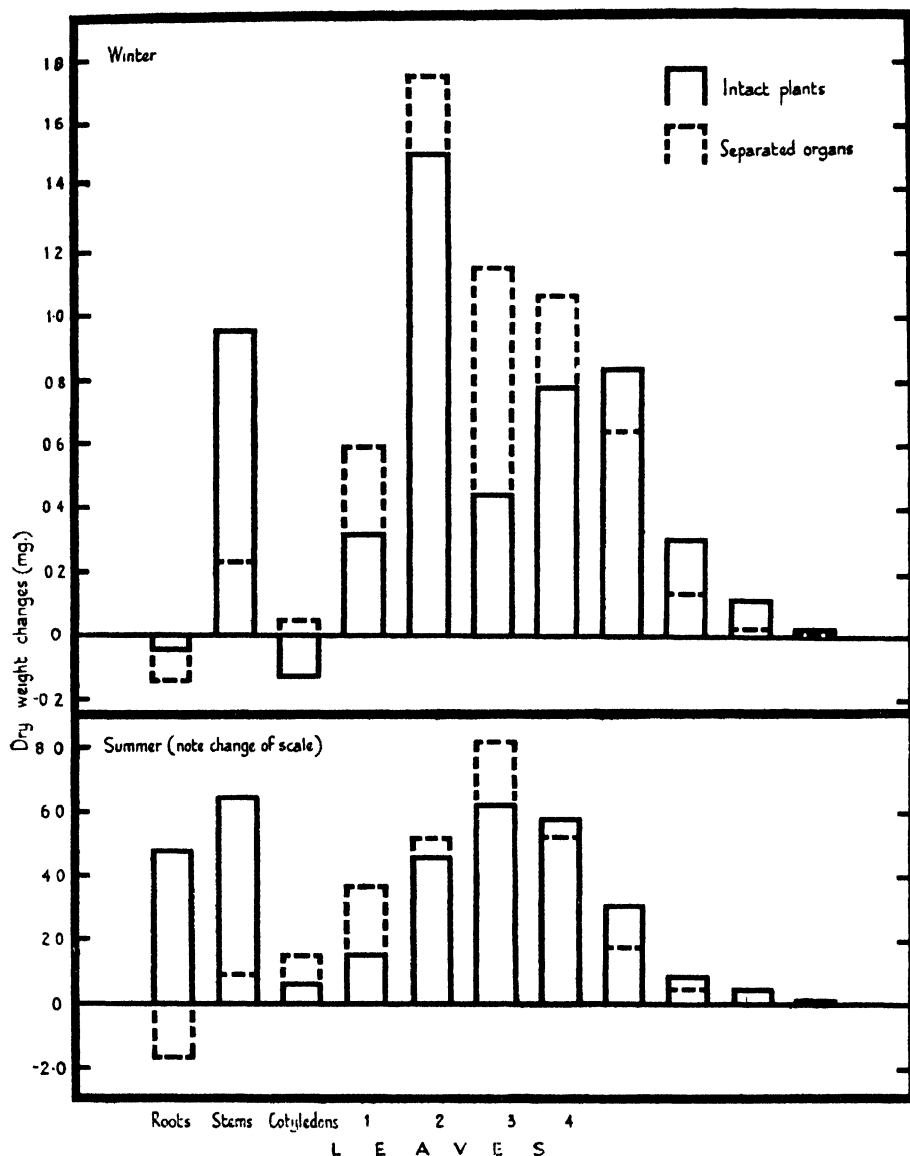


FIG. 8. Absolute changes in dry weight of intact plants and separated organs (corrected) during the afternoon

during the 24 hours from organs suffering a net loss by it is expressed as a percentage of the net assimilation, while that to organs gaining by it is given as a percentage of the dry-weight increase.

In Table VII the data already used in Table VI and Fig. 5 are employed to show the percentage of the total translocation during the 24 hours taking place during the night and during the day.

TABLE VII  
*Translocation during Day and Night as Percentage of Total*  
Winter. Summer.

	Day.	Night.	Day.	Night.
To roots . . .	48	52	93	7
To stems . . .	69	31	77	23
From cotyledons . . .	52	48	74	26
From leaf 1 . . .	81	19	105	5
From leaf 2 . . .	43	57	94	6
From leaf 3 . . .	53	47	64	36
From leaf 4 . . .	63	37	108	208
To leaf 5 . . .	58	42	76	24
To leaf 6 . . .	41	59	75	25
To leaf 7 . . .	53	47	72	28
To leaf 8 . . .	39	61	74	26

These figures confirm the conclusion already reached—that translocation was more active during the day than during the night. Taking the table as a whole, during the short winter day about half of the total translocation during 24 hours took place, while in summer at least three-quarters of the translocation occurred in day-time.

When these percentage changes are converted into absolute values,<sup>1</sup> the results appear as in Figs. 7–11, representing respectively the changes during the four separate periods of the day and during the whole 24 hours. From Fig. 11 it will be seen that in winter about one-third of the translocate went to the root, one-third to the stem, and the remaining third was distributed among the four small leaves. In summer, on the other hand, half the translocate passed to the root, three-eighths to the stem, and only one-eighth went to the younger leaves. As regards the source of this translocate, in both winter and summer about one-third came from the second leaf, and about the same quantity from the third; most of the remainder came from the fourth leaf in winter, from the first leaf in summer. In Table VIII the sources

TABLE VIII  
*Sources and Destinations of Translocate, as Percentages*

Sources	Winter.	Summer.
Cotyledons . . .	11	11
Leaf 1 . . .	6	23
Leaf 2 . . .	33	32
Leaf 3 . . .	29	32
Leaf 4 . . .	21	2
Destinations		
Roots . . .	31	48
Stems . . .	35	38
Leaf 5 . . .	12	8
Leaf 6 . . .	15	3
Leaf 7 . . .	6	2
Leaf 8 . . .	1	1

<sup>1</sup> The mean of the initial log. dry weights of each organ has been calculated for winter and summer, and its antilog. has been used as a basis for conversion of the mean percentage dry-weight changes and translocation values to absolute figures.

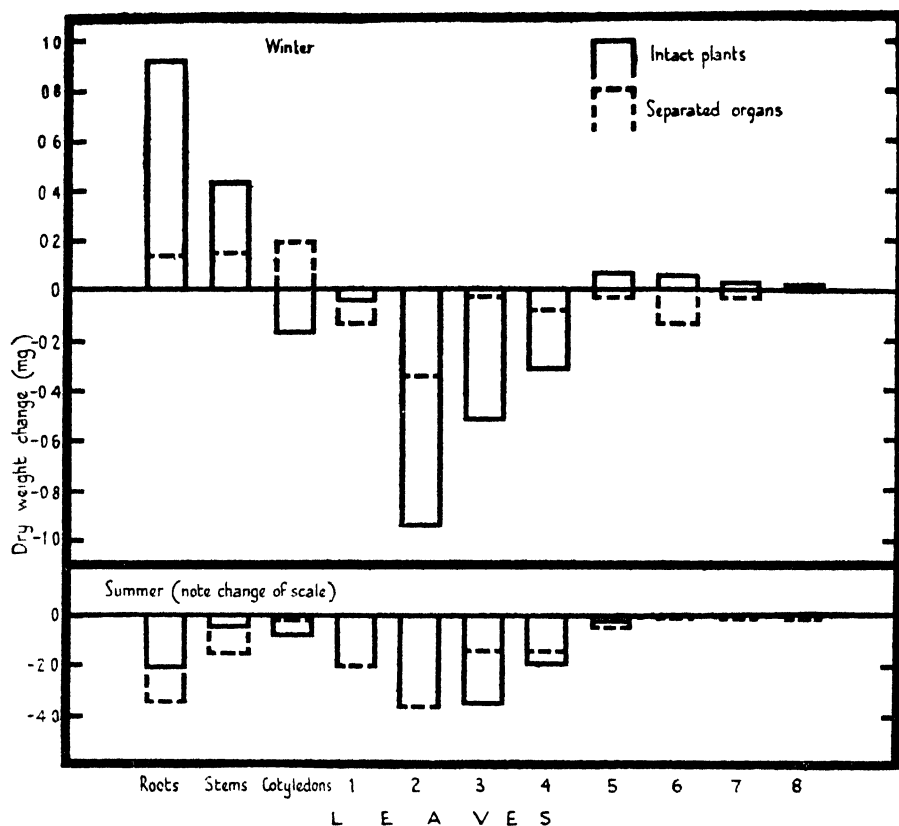


FIG. 9. Absolute changes in dry weight of intact plants and separated organs (corrected) between dusk and midnight.

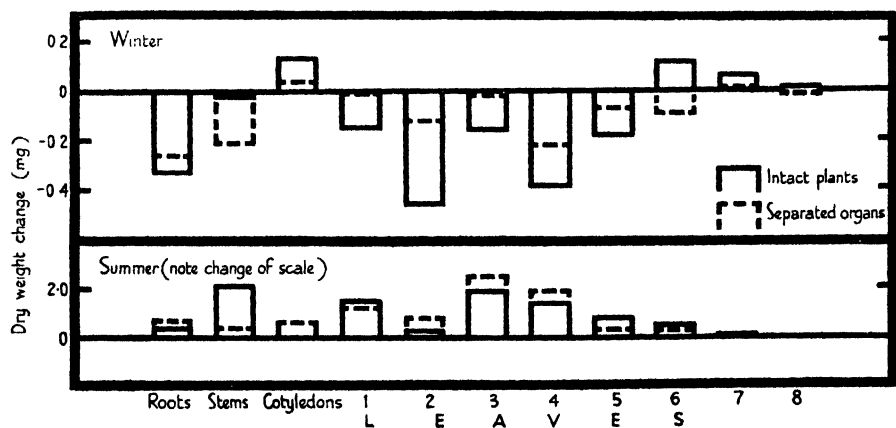


FIG. 10. Absolute changes in dry weight of intact plants and separated organs (corrected) between midnight and dawn.

and destinations of the translocate are expressed as percentages of the total amount.

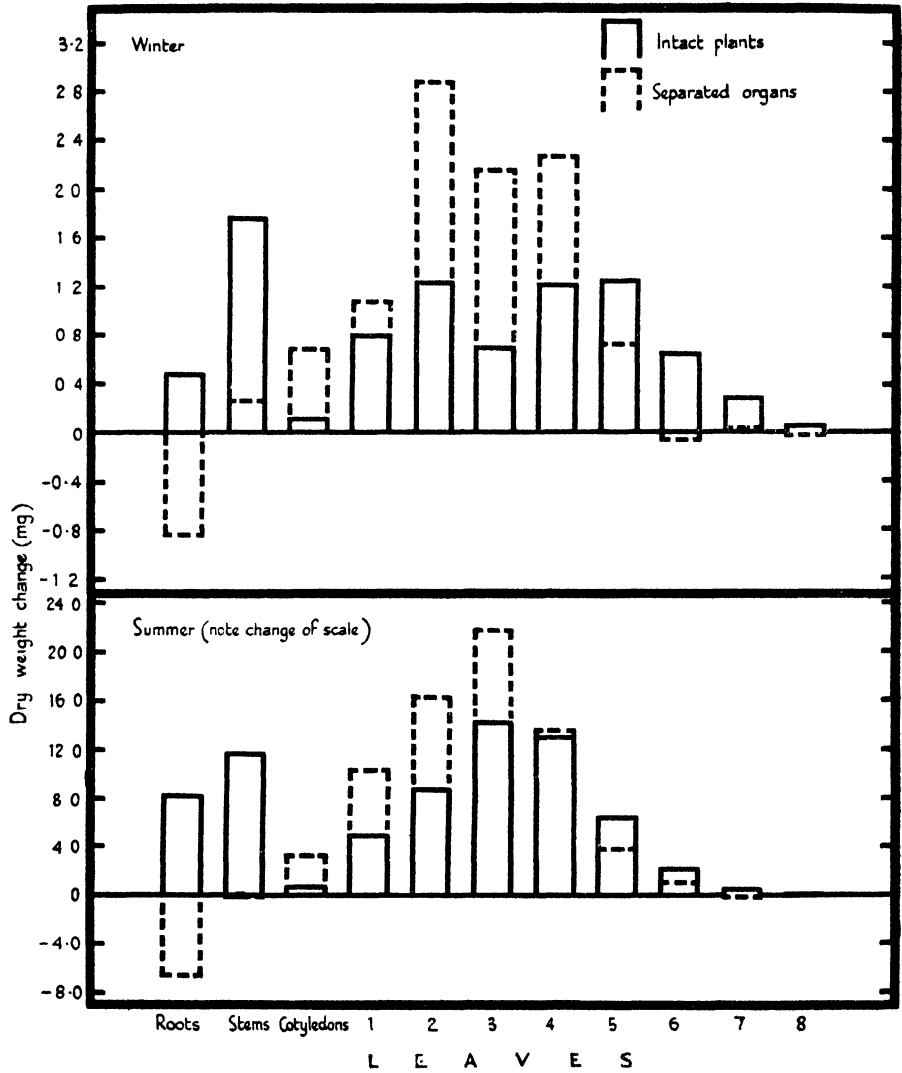


FIG. 11. Absolute changes in dry weight of intact plants and separated organs (corrected) during the 24 hours.

If the absolute amounts of translocate passing from the cotyledons and the four largest leaves are summed and expressed as a percentage of the total dry weight of the plant, one has a measure of translocation rate for the whole plant comparable with the rates of dry-weight change given in the earlier paper (Goodall, 1945). The mean values for winter and summer of these rates of translocation and dry-weight change are shown in Fig. 12. In winter it

will be seen that there is a slight rise in the total translocation in the afternoon, while in summer the highest rate is to be seen before midday. The corresponding figures for the changes during the whole 24 hours in each experiment separately are plotted in Fig. 13. The seasonal increase in both translocation rate and assimilation rate towards midsummer is clear enough.

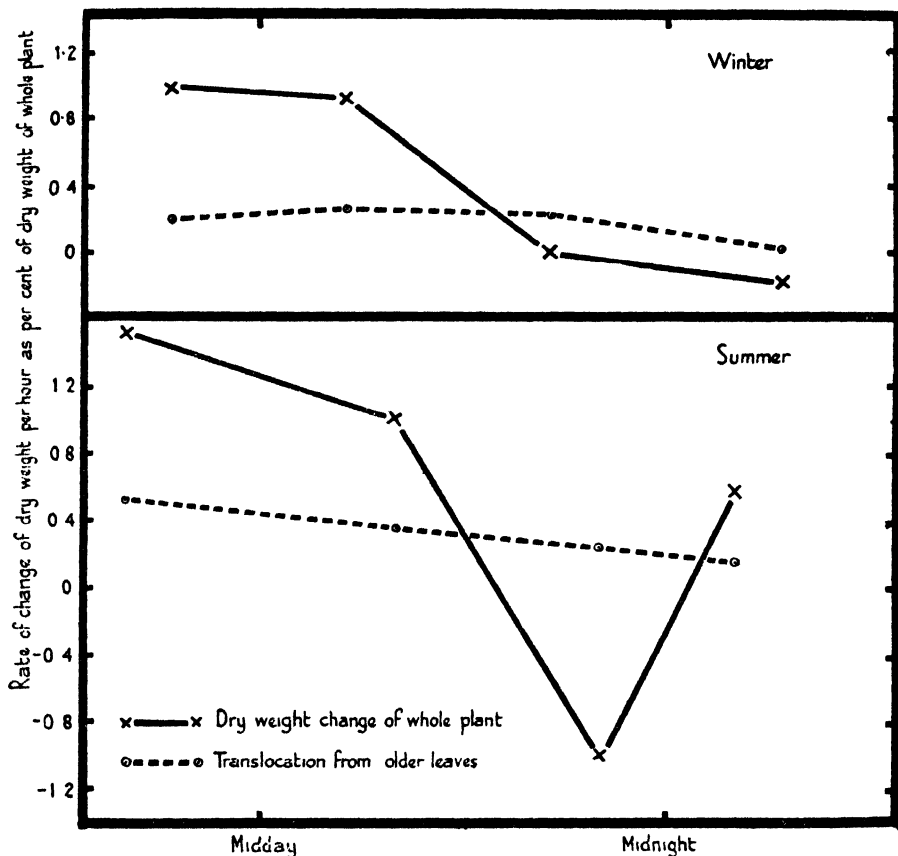


FIG. 12. Course of translocation and of dry-weight change of the whole plant during the day.

T'schesnokov and Bazyrina (1930) have suggested that translocation is dependent upon the amount of 'surplus assimilate' in the leaf. The present data provide an opportunity to test this hypothesis, together with the effect of external factors on translocation rate.

It is difficult to understand what is meant by 'surplus assimilate'. It cannot be the increase in dry weight of the leaf over its initial weight, for, in addition to labile materials (including reserves), this increase includes assimilate which has already been incorporated into the substance of the leaf—as cellulose, protoplasm, and so forth. The amount of labile substance which might be what is intended by the phrase 'surplus assimilate' can only be

assessed by detailed analysis of the leaf tissue. It is, however, a fair assumption that in the two samples taken at successive dawns labile substances form much the same proportion of the leaf material. In the absence of other information we may further assume that the increase in the non-labile material of the leaf takes place continuously at a constant rate throughout the 24 hours. The non-labile material in the leaf at the end of each period, together with the proportion of labile material present at dawn, has accordingly been calculated on this basis, and the excess of the actual leaf dry weight over this figure has been taken as the amount of 'surplus assimilate' in the leaf.

The cotyledons and first four leaves were grouped together, and in each period and experiment the initial amount of 'surplus assimilate' and the mean rate of translocation per hour, each expressed as a percentage of the initial dry weight of these leaves, together with their mean assimilation rate and the mean light intensity and temperature, have been used in an analysis of covariance; this is set out in Table IX.

TABLE IX

*Analysis of Covariance: Translocation Rate from Older Leaves, eliminating 'Surplus Assimilate', Assimilation Rate, Light Intensity, and Temperature*

	<i>n.</i>	Sum of squares.	Mean square.	<i>F.</i>	<i>P.</i>
Time of day . . . . .	3	0.1044	0.0348	—	—
Time of year . . . . .	22	2.9242	0.1329	2.37	< 0.01
Partial regression on 'surplus assimilate', eliminating assimilation rate, light, and temperature . . . . .	1	0.3918	0.3918	7.00	0.01–0.05
Partial regression on assimilation rate, eliminating 'surplus assimilate', light, and temperature . . . . .	1	0.8932	0.8932	15.91	0.01
Partial regression on light, eliminating 'surplus assimilate', assimilation rate, and temperature . . . . .	1	0.2351	0.2351	4.20	0.01–0.05
Partial regression on temperature, eliminating 'surplus assimilate', assimilation rate, and light . . . . .	1	0.0768	0.0768	1.37	> 0.05
Multiple regression on 'surplus assimilate', assimilation rate, light, and temperature . . . . .	4	1.9457	0.4864	8.70	< 0.01
Error . . . . .	58	3.2491	0.0560	—	—

The partial regression on temperature in Table IX is not significant, but it was found that if the same partial regression was computed for the means for the different experiments it was significant ( $F = 4.89$ ;  $n_1 = 1$ ,  $n_2 = 19$ ,  $P = 0.01-0.05$ ). This discrepancy may be because most of the variation in temperature occurs between times of day and times of year, and not in the interaction term, from which the partial regressions in the table are derived.

An alternative explanation is that it may be due to a correlation of translocation rate with another factor that has not been taken into account, itself correlated with temperature as between different times of year but not in the interaction of time of year with time of day. The former explanation seems simpler, and the two regression coefficients (that from the time-of-year differences and that from the interaction term) do not differ significantly,

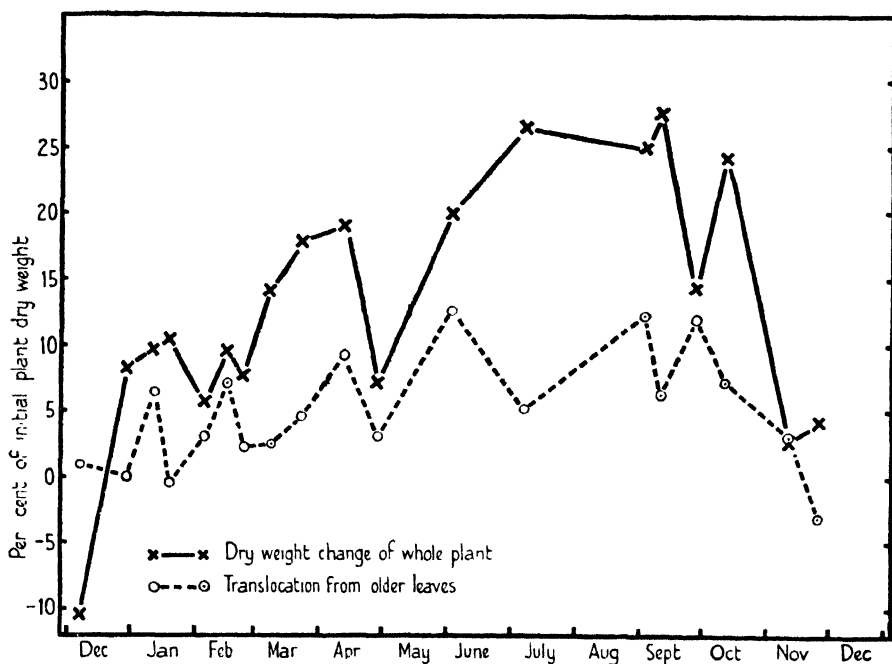


Fig. 13 Translocation and dry-weight increase of the whole plant during 24 hours, at different seasons of the year.

although they differ in sign. In any case, it seemed desirable to include temperature in the multiple regression as well as the other three independent variables. The regression equation is:

$$Y = 0.0530S + 0.2462A + 0.00063L - 0.0289T,$$

where  $Y$  is the translocation rate and  $S$  the assimilation rate (both per cent. per hour),  $A$  the 'surplus assimilate' (per cent. of leaf dry weight),  $L$  the mean light intensity (foot-candles), and  $T$  the mean temperature ( $^{\circ}\text{C}.$ ), each variable being measured from its mean. The positive partial regression on assimilation rate cannot be regarded as definitely indicating a relation between the two processes, for in the method of calculation adopted a fortuitous error in the final weight of the detached organs would cause errors in the same direction in both the estimated translocation and assimilation rates. No such relation exists, however, between the estimates of initial amount of 'surplus assimilate' and translocation rate, so that the relation found must be regarded as real.

The regression on light only just passes the level of significance and, though suggestive, cannot be regarded as fully established.

When the effect of the four independent variables has been eliminated, the differences in translocation rate between the four periods of the day, previously highly significant ( $F = 10.18$ ;  $n_1 = 3$ ,  $n_2 = 62$ ,  $P < 0.01$ ), becomes non-significant—in other words, the differences between the four periods may be explained adequately on the basis of the different mean temperatures, light intensities, quantities of 'surplus assimilate' present in the leaves, and perhaps assimilation rate. On the other hand, the differences between the experiments carried out at different times of year remain highly significant, showing that other factors are also operating here to cause differences in translocation rate.

The data also provide an opportunity to test the hypothesis often put forward (see p. 314) that the accumulation of assimilate in the leaf inhibits further assimilation. For this purpose only the afternoon figures (for the same organs) were used, since before midday the mean 'surplus assimilate' value would be largely dependent upon the assimilation rate during the same period. Here the primary correlation was positive, but it was evident that seasonal differences in environment would obscure any effect of internal factors. Therefore a partial correlation coefficient was calculated, eliminating the temperature and light intensity during the period in question. This coefficient was  $-0.134$  ( $n = 15$ ), i.e. negative and non-significant. Calculation of a multiple regression led to a similar conclusion; the regression equation was:

$$X = 0.00499L + 0.0404T - 0.0224A,$$

where  $X$  is the mean assimilation rate (per cent. per hour),  $L$  the mean light intensity (foot-candles),  $T$  the mean temperature ( $^{\circ}\text{C}.$ ), and  $A$  the 'surplus assimilate' (per cent.), again measured from their means. From the analysis of variance in Table X it will be seen that the additional variance ascribable to the effect of the 'surplus assimilate' is negligible:

TABLE X  
*Analysis of Variance: Partial Regression of Assimilation Rate  
on 'Surplus Assimilate'*

	<i>n.</i>	Sum of squares.	Mean square.
Regression on $L$ and $T$ , not eliminating $A$	2	3.43	1.72
Regression on $A$ , eliminating $L$ and $T$	1	0.07	0.07
Remainder	15	5.20	0.35
Total	18	8.70	

Thus, these data cannot be regarded as supporting the hypothesis of Saposchnikoff (1890) and others that accumulation of assimilate reduces the assimilation rate. On the other hand, these negative results cannot be regarded as at all conclusive. It is important to note that the assimilation rate during a period enters into the estimation of the mean amount of 'surplus assimilate'



during this period, and the effect of this would tend to nullify any correlation in the direction postulated, if it existed.

#### SUMMARY

Assimilation, respiration, and translocation were studied in the tomato plant, at a stage when it had developed eight leaves, by following during the 24 hours the dry-weight changes both in the intact plant and in the separated organs.

Turgid, separated leaves if fully exposed to light may assimilate more than similar leaves attached to the plant.

When separated leaves were supported in such a manner that they were subjected to the same conditions of mutual shading as on the plant, they assimilated less than attached leaves, even though they showed no signs of wilting. Wilted leaves assimilated much less than turgid leaves.

Root respiration in the evening was more active in summer than in winter, in spite of the similarity of the mean temperatures.

Detached leaves lost dry weight more rapidly from dusk to midnight than from midnight to dawn.

In the detached leaves, assimilation before midday was more rapid in summer than in winter; but for the afternoon the winter figure was higher.

The respiration rate was highest and the assimilation rate lowest in the very young leaves. The assimilation rate in successive leaves increased from the youngest down to the leaf which had reached about half its final length and then remained fairly constant.

In all parts of the plant, translocation was more rapid by day than by night; on average it was about twice as rapid.

At all times translocation was proceeding from the cotyledons and the first four leaves to the stem, root, and the four younger leaves.

The fifth leaf, with a length of 7 cm., obtained by translocation about one-third of its increment in dry weight over the 24 hours.

Translocation during the day was more rapid in summer than in winter, but the greater amount of translocation in summer, as in the case of assimilation, is to be ascribed rather to the longer day than to an increased rate.

About half the material translocated to the root during the 24 hours was lost in respiration. In the case of the stem, on the other hand, assimilation and respiration approximately balanced, and its increase in dry weight corresponded to the amount translocated to it.

In winter, one-third of the material translocated from the older leaves passed to the root, one-third to the stem, and one-third to the young leaves. In summer, the proportions were respectively half, three-eighths, and one-eighth.

Evidence is adduced that the rate of translocation is affected by the amount of 'surplus assimilate' present in the leaf. It is probable that light intensity and temperature are also concerned, though this is not established. These three factors, together perhaps with the assimilation rate at the time, account

satisfactorily for all the variation in translocation rate with time of day, but not for all the variation between translocation rates found at different seasons.

There is no evidence that the amount of 'surplus assimilate' present affects the assimilation rate.

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# Anatomical Observations on a New Species of *Batrachospermum*

BY

KATHILEEN M. DREW

(Department of Cryptogamic Botany, University of Manchester)

With Plate VIII and seventeen Figures in the Text

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## 1. INTRODUCTION

WHILE attempting to compile a list of the species of *Batrachospermum* which occur in the British Isles, two species growing in the river Wye as it flows through Chee Dale and Millersdale, Derbyshire, have been under observation. The larger species agrees with the descriptions of *B. ectocarpum* Sirodot, and as far as the writer is aware this is the first record of the occurrence of this species in the British Isles. The smaller species appears not to have been described previously and is now named *B. fruticulosum*.

From a morphological viewpoint *B. fruticulosum* is of considerable interest, observations having shown that secondary developments result in the establishment of an adult axis which in transverse section resembles that of a floridan alga with the multiaxial type of construction, such as *Nemalion*, rather than that of alga with the uniaxial construction, of which *Batrachospermum* is so often quoted as the example. This form of adult axis has been found in two other species of the genus, as has been reported briefly already (Drew, 1945). The stages by which this secondary axial structure is reached are now described and illustrated in detail for the first time.

Another point of interest of this new species, *B. fruticulosum*, is that it produces adventitious branches of unlimited growth very freely and even abundantly at some seasons of the year. They may develop from either the corticating filaments or cells of the primary and secondary branches of limited growth.

The Chantransia-stage has been found on stones in the river and also growing endophytically in the adult plant. On one occasion (June, 1945) the quantity of the endophytic Chantransia-stage was small, but on another occasion (November, 1944) the extent of the growth was very spectacular and a large number of branches of the adult type were arising from it. So numerous were

these adult branches that when viewed under a binocular microscope they appeared to cover the adult thallus.

After the Latin diagnosis and a description of *B. fruticosum* a further section is devoted to the axial structure and the adventitious branches.

## 2. DESCRIPTION OF PLANT

*Fruticosum*, ad 7 cm. longum; fuscum aut nigrum; exsiccatum nigrum; profuse ramosum, sed multi rami breviores; verticilli juveniles contigui, partes thalli inferiores cylindraceae; cellulae exteriores elliptico-globosae, interiores ellipsoideae; pili rarissimi; filamenta interverticillaria abundantia; cellulae cylindraceae; cortex interverticillarius, primo celeriter crescens, tum ad aliquantam crassitudinem ( $450\ \mu$ ) perveniens, tandem cellulas axis primarii ita penetrans ut vix ullum vestigium axis primarii maneat.

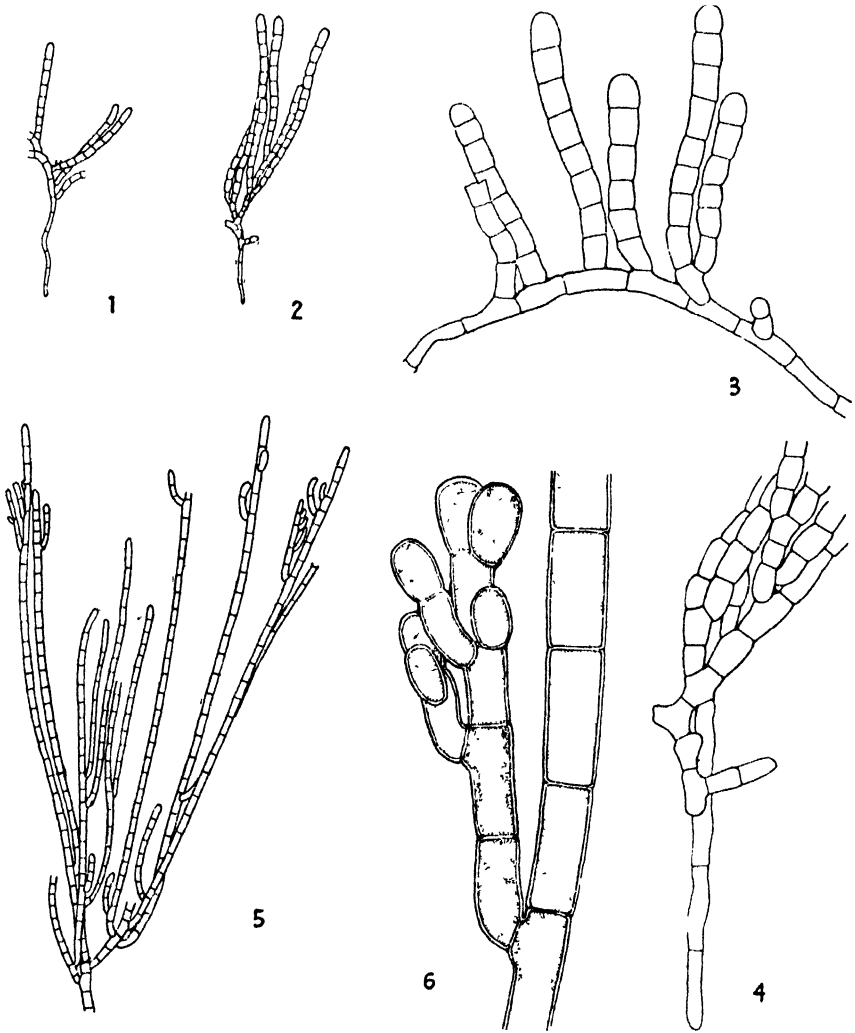
*Chantransia*—forma viridis, aut in lapidibus submersis habitans, aut parenti adhaerens et endophytica, circa 2 mm. longa, cellulae cylindraceae diam.  $10.5\text{--}13.0\ \mu$ ; monosporangia diam.  $8.0\text{--}10.5\ \mu$ .

Thalli monoeci; spermatangia (diam.  $5\ \mu$ ) et carpogonia in verticillis et in filamentis interverticillariis, compluria carpogonia in singulis verticillis; trichogynum juvenale ovoideum, postea ellipsoideum; glomeruli fructiferi diam.  $100.0\ \mu$ , ad sex in singulis verticillis, ex ordine maturescentes, plerumque in peripheria verticillorum, aliquando exserti.

Hab. in lapidibus in flum. Wye, Derbyshire, Eng.

Type specimen, Drew, no. 1610b, in the Manchester Museum.

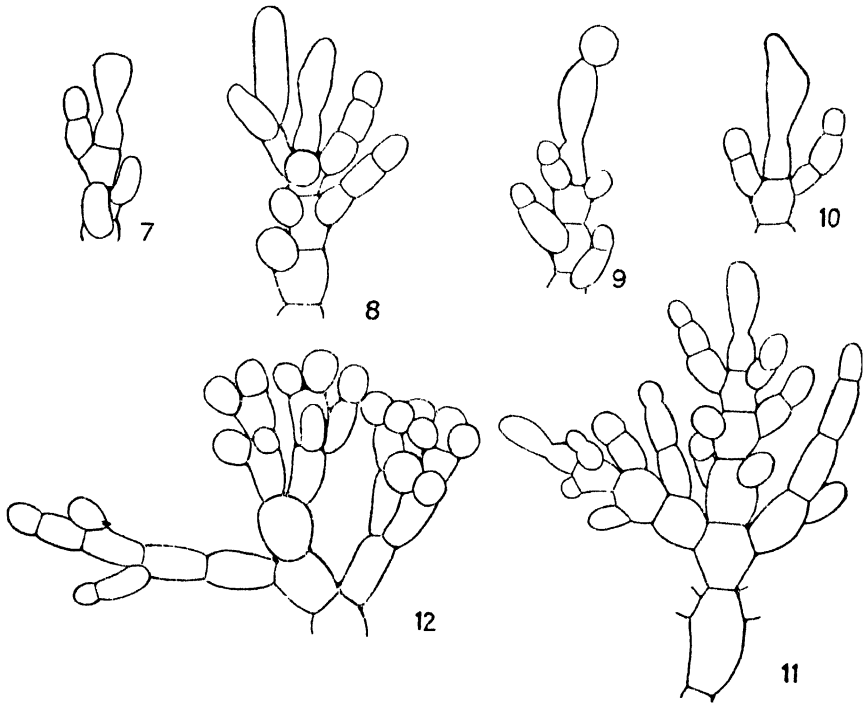
*Plants* bushy, up to 7 cm. long; mucilaginous; very dense dark brown to black, young plants having greenish tinge in transmitted light, dried specimens black; crystals occurring in very old parts of thallus; probably comparatively long-lived, known to occur in March, June, July, November (Pl. VIII, Figs. 1, 2, and 3). *Thallus* varying in diameter from approximately  $200\ \mu$  at the apex to  $1,500\ \mu$  and more at the base; profusely branched. *Branches of unlimited growth* arising irregularly on all sides of the main axis, spreading, tips pointed; of two types, some growing long and equalling the parent axis but the majority remaining shorter, the latter in the apical zone of old plants (Pl. VIII, Fig. 1). *Primary branches of limited growth* occurring in whorls of six; sparsely branched; cells bead-like, outermost sub-spherical, inner ellipsoidal; plastids parietal; hairs extremely rare, the two seen being short with bulbous base. *Whorls* of primary branches of limited growth contiguous for a short distance behind apex, then sufficiently separate over a varying length to be visible to the naked eye, ellipsoidal-spherical; basal portions of plants with continuous cylindrical outline (Pl. VIII, Figs. 1, 2, and 3). *Secondary branches of limited growth* arising in profusion from the cortex of the central filament; cells rectangular; branching sparse and from basal part, somewhat resembling the *Chantransia*-stage (Pl. VIII, Figs. 8, 11). *Cortication* developing very rapidly, soon reaching several layers in thickness; in the basal region the central strand may reach a diameter of  $450\ \mu$ ; inner layers compact but outer layers looser, giving rise to branches of limited and unlimited growth



TEXT-FIGS. 1-6. 1 and 2. Small portions of young endophytic *Chantransia*-stage ( $\times 93$ ). 3. Portion of stoloniferous endophytic *Chantransia*-stage from which free-growing branches are arising ( $\times 375$ ). 4. Base of Fig. 2 enlarged to show development of rhizoidal branch ( $\times 375$ ). 5. Saxicolous *Chantransia*-stage ( $\times 93$ ). 6. Monosporangial branchlet on similar plant ( $\times 625$ ).

(Pl. VIII, Figs. 5-11); innermost layer giving rise to branches which penetrate and ultimately replace the axial cells (Pl. VIII, Figs. 6, 7, 8, 9, 12); persisting after branches of limited growth denuded (Pl. VIII, Figs. 15, 16, 19). *Chantransia*-stage occurring in March, June, July, and November on stones (Text-fig. 5) or endophytically on parent (Pl. VIII, Fig. 19); green, up to 2 mm. high; branching sparse and confined to basal parts, irregular, often from two consecutive cells and on the same side of the axis, branches arising at an acute angle; cells rectangular in optical section,  $10.5-13.0\mu$  in diameter, on the

average  $1\frac{1}{2}$  times to twice as long as broad, apical cells shorter in proportion; cells of endophytic Chantransia-stage from  $8\mu$  in diameter and relatively shorter; one or more plastids, parietal or band-shaped, usually the latter at the basal end of the cell; hairs absent; monosporangia occasional (Text-fig. 6),  $8.0-10.5\mu$  in diameter. *Reproduction*. Usually fertile; monoecious, reproductive organs usually occurring in separate whorls but sometimes in close



TEXT-FIGS. 7-12. 7 and 8. Young carpogonia before fertilization. 9. Spermatium attached to trichogyne of carpogonium. 10. Irregularly shaped trichogyne characteristic of mature but unfertilized carpogonia. 11. Carpogonial branch with lateral as well as terminal carpogonia. 12. Axial region of branch of unlimited growth with spermatangia. (All  $\times 850$ .)

proximity. *Spermatangia* occurring in terminal clusters, borne terminally and laterally on the cells of the fertile branchlets (Text-fig. 12); frequently developing on the secondary branches of limited growth; sometimes developing on the lateral branchlets of the carpogonial branches and bracts subtending the cystocarps; spermatangia on the average  $5\mu$  in diameter. *Carpogonial* branches several in each whorl, often in the outer part and sometimes giving rise to lateral carpogonia as well as the terminal one (Text-fig. 11), frequently developing on the secondary branches of limited growth; of medium length, branched towards apex; trichogyne ovoid when young (Text-fig. 7) becoming ellipsoidal (Text-figs. 8 and 9) when older and often slightly asymmetrical, if unfertilized developing into very irregular shapes (Text-fig. 10); between  $16.0\mu$  and  $24.0\mu$  in length when approaching maturity. *Cystocarps* develop-



ing consecutively, hence young shoots show only one cystocarp per whorl whilst older shoots may have as many as six per whorl; at this stage numerous cystocarps also on the secondary branches of limited growth; occurring in outer layers and frequently exerted; up to  $100\ \mu$  or more in diameter.

The distinguishing features of *B. fruticosum* are its bushy growth, dark colour, and comparatively small size. The dense growth of secondary branches of limited growth, the thick persistent axial strand, and the tendency to form adventitious branches of unlimited growth are also characteristic.

*B. fruticosum* belongs to the Helminthoidea section of the genus as delimited by Sirodot (1884) on account of the number of small gonimoblasts and the ovoid-ellipsoidal shape of the trichogyne. This species differs from *B. Boryanum* and *B. anatinum* which also belong to this section in that it is monoecious. From the monoecious examples of the polygamous *B. anatinum* it can be distinguished by its rich branching and the abundance of cystocarps on the secondary branches of limited growth. The other monoecious species of this section of *Batrachospermum* are *B. helminthosum*, *B. Crouanianum*, and *B. distensum*. *B. fruticosum* is easily distinguished from *B. distensum* by the numerous secondary branches of limited growth. From *B. helminthosum* it is distinguishable by the frequent development of carpogonia on the secondary branches of limited growth as well as by lateral carpogonia on the carpogonial branches. The Chantransia-stage of *B. helminthosum* has hairs unlike the Chantransia-stage of *B. fruticosum*. Like *B. Crouanianum*, *B. fruticosum* is a smaller species, but spermatangia never occur on either the carpogonial branches or the secondary branches of limited growth of the former species, as they do in the latter.

The occurrence of the endophytic Chantransia-stage may be due to the development of carpospores more or less *in situ*. This has not been observed in this species of *Batrachospermum*, but Fritsch (1945, p. 455) states that 'germinating carpospores are not uncommonly found entangled among the threads of the parent', and Sirodot (1884, p. 189) records similar observations. Alternatively or in addition, this endophytic Chantransia-stage may originate from transformation of the corticating filaments, examples suggestive of this having been seen. Superficial cells of axes denuded of radially growing branches also occasionally grow out into short filaments bearing monosporangia, similar to those which develop on the Chantransia-stage. The endophytic Chantransia-stage is similar to that growing on stones apart from the basal regions. The lower cells of the erect filaments give rise to down-growing rhizoidal branches and (Text-figs. 1, 2, and 4) stoloniferous filaments are also common (Text-fig. 3). It provides good material for the study of the origin of the branches of the adult type (Pl. VIII, Figs. 19, 20). The change from one type of axis to the other, while marked, is gradual. The first central cells are intermediate in form between cells of the Chantransia-stage and typical adult axial cells. Likewise the first formed whorls of limited growth are very simple and have no cortication of the lowest internodes except possibly ultimately by growth from higher nodes.

It has been stated (Mühldorf, 1937) that there are no pit-connexions in *Batrachospermum* but they are quite obvious in this species.

### 3. THE DEVELOPMENT OF THE 'THICKENED' AXIS AND ADVENTITIOUS BRANCH FORMATION

The occurrence of plants such as that of Pl. VIII, Fig. 1, characterized by evenly cylindrical basal portions and apical parts having the appearance typical of *Batrachospermum* clearly called for closer attention. The difference between the apical and basal parts of the plant is so marked that it would be easy to mistake the upper part for an epiphyte on the other. Moreover, transverse sections taken singly in the evenly cylindrical basal and in the noded apical regions would support this point of view, for the former shows a pseudo-parenchymatous central region with filaments radiating from it (Pl. VIII, Fig. 11) and the latter a central cell, surrounded by corticating filaments and primary as well as possibly secondary, branches of limited growth (Pl. VIII, Fig. 5). However, a series of sections passing from the apex of a shoot downwards shows that both types of shoot and both types of axial structure belong to one plant, the structure in the basal portion representing a secondary development from the primary structure of the apical portion. Indeed such a 'thickened' condition occurs in comparatively young axes.

The early stages in the development of the axis of *B. fruticosum* agree with those already described for various species of the genus. The dome-shaped apical cell cuts off segments, from each of which whorls of six branches of limited growth develop very quickly before the segment has elongated to any extent. Usually one such branch of each whorl gives rise to a branch of unlimited growth from the upper side of its basal cell, and this branch repeats the structure of the main axis. In addition corticating filaments arise from the lower side of the basal cell of each of the six branches of limited growth. These grow downwards, keeping in contact with the outer wall of the main axial filament of cells. In this particular species these corticating filaments develop early, grow rapidly and vigorously so that they soon pass the adjacent node and add to the cortication already existing in the lower internodes. They also branch extensively, the branches developing into either further corticating filaments or filaments which penetrate and ultimately replace the central filament of cells. Still other branches develop into radially growing filaments of limited growth with assimilatory and reproductive functions and occasionally others become adventitious branches of unlimited growth. From this it can be seen that the corticating filaments play a very important role in the morphology of this plant, ultimately producing a comparatively elaborate thallus.

Before describing the penetration of the central filament of cells, which is a newly discovered feature in this genus and very uncommon throughout the Florideae, it should be pointed out that the cells of the secondary branches of limited growth are much smaller in diameter than the primary ones and the cells are cylindrical and not ellipsoidal (Pl. VIII, Figs. 5 and 6). Although

reminiscent of a Chantransia-stage, they are often fertile, bearing both spermatangia and carpogonia.

When the cortex is several layers thick, branches of the innermost layer of filaments penetrate the cells of the central filament. This usually takes place first in the neighbourhood of a node, but later branches may enter at any level.



TEXT-FIGS. 13 and 14. 13. Centre of axial strand in transverse section. Wall of central cell in fragments and in lumen of cell invading filaments in section. Young one showing connexion with cell of cortical filament. 14. Transverse section of portion of wall of central cell of axis of Pl. VIII, Fig. 6 showing the very early stage of the penetration of the wall by an outgrowth from a cell of cortical filament (Both 850.)

A very early stage in this penetration is shown by the example of Text-fig. 14 (a portion of the central cell of Pl. VIII, Fig. 6), a bulge from a cortical cell having broken through the wall of the central cell. By means of focusing to different levels the wall is found to be intact both above and below the penetration. An older example is figured on Pl. VIII, Fig. 12. Here the penetrating filament is already four-celled and growing across the lumen of the central cell. The pit-connexion with the parent cell of the corticating filament is easily seen. Several other invading filaments occur in this section, cut either obliquely or longitudinally and only fragments of the cell wall remain. A still later stage in the development of an invading filament is shown in Text-fig. 13. Once a central cell has been penetrated it is not long until it is full of filaments (Pl. VIII, Figs. 8 and 9). The internal filaments are usually narrower than the

corticating filaments, and while the first-formed grow in a downward direction subsequent filaments may grow either obliquely or transversely in older internodes when the wall of the central cell is mostly destroyed. Ultimately almost all trace of the axial filament of cells is lost, as in Pl. VIII, Fig. 11, and as the transverse walls between neighbouring central cells break down also, the penetrating filaments pass from one cell to the next.

The 'solid' structure as shown in Pl. VIII, Fig. 11, is to be found through almost the entire length of the adult axis, but just above each node there are usually remnants of the wall or lumen of the central axial cell or occasionally both as in Pl. VIII, Fig. 10. This is due to the fact that the upper end of each cell of the central filament has a much smaller diameter<sup>1</sup> than the basal end, and being narrower is filled more easily and a 'solid' structure results more quickly. The basal end of each cell, being so much greater in diameter, is seldom filled completely by penetrating filaments, and portions of the cell wall remain.

A similar form of adult axis has been found to develop into other species of *Batrachospermum*. One of these, collected in a tributary of the river Wye in Monkdale in July 1937, was remarkable on account of the large diameter of the thallus. Although the plants were 4 in. long, the growing tips had decayed away already. The central strand is smaller in proportion to the whole axis than in *B. fruticosum* but is almost continuously 'solid', only rare indications of the central cells occurring immediately above a node (Drew, 1945). Identification of this species has not been possible as only male reproductive organs have been seen. It recalls Kützing's description and figures of *B. giganteum* Kützing, however, given by Sirodot as a synonym of *B. helminthosum* Sirodot.

The second species was collected from a trough at Hawkshead Hill, Westmorland, in May 1942 and April 1945, and has been identified provisionally as *B. distensum* Kylin.<sup>2</sup> It is noteworthy on account of its small central strand and the luxuriance of the branches of limited growth. The central filament is heavily corticated, but has very few secondary branches of limited growth.

Records of the penetration of axial cells by filaments from pericentral cells have been given by Phillips (1926) for *Ceramium rubrum* and Feldmann-Mazoyer (1940) for species of the same genus (particularly *C. tenuissimum* and *C. diaphanum* v. *zostericola* f. *minuscula*). Feldmann-Mazoyer found that the axial cell might be completely filled by such rhizoids, but considers the condition exceptional and probably due to the previous death of the cell. Both writers consider the penetrating filaments as analogous to tyloses. In the case

<sup>1</sup> Sirodot (1884) mentions this peculiar shape of the central cell and suggests it is due to the comparative rates of elongation and transverse expansion of the cell, coupled with the rate of development of the corticating filaments. This explanation can only be verified by direct observation but is very probably correct.

<sup>2</sup> *Batrachospermum distensum* has not been previously recorded from England, but a specimen in the British Museum of Natural History and collected from Blessington, Co. Wicklow, has been identified by Skuja as such.

of *B. fruticulosum* the state of the protoplast suggests that the cell is alive at the time of the first penetration, but there has been no more detailed determination of its physiological condition. In the species of *Batrachospermum* mentioned, the penetration of the central filament of cells appears to be part of the normal process of secondary development and not an exceptional event as in species of *Ceramium*.

During the examination of various collections of *B. fruticulosum* it became obvious that the formation of adventitious branches is not an uncommon occurrence in this species. One or two plants collected during November were found to be literally covered by young adventitious branches in addition to the Chantransia-stage, described already. Some of these branches arise from the cortical filaments, some from the primary and secondary branches of limited growth.

As an example of the latter, the reader is referred to Pl. VIII, Fig. 13, a transverse section of a young but well-developed part of an axis, showing two young branches of unlimited growth arising quite near the periphery of the thallus. One of these young branches is in median section (although not entirely in one focal plane) and only the tip of the other is at this level. The former is shown at a higher magnification in Pl. VIII, Fig. 14. The branches in question originate from either a primary branch of limited growth or else filaments which are corticating it. It is certain, however, from direct observation that adventitious branches can arise from both primary and secondary branches of limited growth.

Many of the adventitious branches found on the plants collected in November arise from the cortex of the central axis. The thallus has been worn back to the axial strand (Pl. VIII, Figs. 15 and 16), the inter-filamentous spaces of the peripheral layers of which have been invaded by bacteria and blue-green algae. At the same time, the cells of these outermost layers are in a state of rejuvenescence, as is obvious from the deep staining of the cell contents, and many of the cells are beginning to grow out in a radial direction. In places they are giving rise to branches of unlimited growth as in Pl. VIII, Figs. 15 and 16. The very young branch apex of Pl. VIII, Fig. 16 (indicated by an arrow) is shown at a higher magnification in Pl. VIII, Fig. 17, and reproduced again as Text-fig. 15. Here the pit-connexions are shown from the apex of the branch downwards as far as they can be traced in this section, that is well into the cortex. A similar but older branch of unlimited growth arising from the cortex is shown in Pl. VIII, Fig. 15. When such adventitious branches arise deep in the cortex, branches of limited growth are not formed from the first segments. A further example of adventitious branches arising from cortical filaments is given in Text-fig. 16. As a contrast, Pl. VIII, Fig. 18, shows two young shoots of unlimited growth, which have developed from the cortical filaments of material which is not denuded as in the previous examples quoted. It will be noticed that one is a branch of the other and arises directly from the axial cell. This has been seen to happen frequently among these adventitious branches.

The position of the young adventitious branch of Text-fig. 17 as well as the appearance of the branch suggests that it has replaced a carpogonium. While some of the details of the present records may be new, the fact that such branches occur in the genus *Batrachospermum* was recorded by Sirodot



TEXT-FIGS. 15, 16, and 17. 15. Adventitious branch of Pl. IX, Fig. 17, drawn to show pit-connexions as far as can be traced in section ( $\times 625$ ). 16. Adventitious branch arising from cortical filaments of denuded axis ( $\times 850$ ). 17. Adventitious branch in terminal position on carpogonial branch ( $\times 850$ ).

(1884). Giving them the name 'proliferation', he reported having seen them on thalli of *B. densum* Sirodot, *B. Decaisneanum* Sirodot, *B. pyramidale* Sirodot, *B. pygmaeum* Sirodot, and *B. Dillenii* Bory, developing from both the cortical filaments and the branches of limited growth.

#### 4. DISCUSSION

Although widely distributed and often easily accessible, the freshwater Florideae have for some decades not received much attention apart from the work of Skuja (1931, 1933, 1934). Yet there is little doubt that this field of study would be a profitable one in many respects, not least in providing clearer concepts of the genera concerned. For example, *Batrachospermum*

is usually quoted as an example of the simplest type of uniaxial construction, but while this is true of some species, the observations made on a new species of *Batrachospermum* at various times of year and recorded in the preceding pages, show that considerable elaboration of the primary construction and a high degree of internal differentiation can be reached in this genus. There is some reason for believing that this more elaborate construction is not rare in the genus as two other species are known to be essentially similar to *B. fruticulosum* in this respect.

The elaboration of the primary structure is brought about by the rapid and vigorous growth of the filaments which originate from the basal cells of the primary branches of limited growth and corticate the primary central filament. Branches of the first-formed corticating filaments function either as further corticating filaments or penetrate the primary central filament producing a homogeneous axial strand of longitudinally running filaments. This axial strand probably has mechanical and physiological functions; its undoubted biological function is referred to later. Around this axial strand is a wealth of radially arranged branches of limited growth, themselves branches of the corticating filaments, with photosynthetic and reproductive functions similar to those of the primary branches of limited growth. Although having similar functions, the primary and secondary branches of limited growth differ in appearance. Lastly, adventitious branches of unlimited growth may develop from the corticating filaments.

As is well known, most of the Florideae have a vegetative construction conforming to one or other of two types, called the uniaxial and multiaxial. In their simple condition each type is easily distinguishable, but elaboration and secondary developments may obscure the underlying plan. As a result of secondary changes it sometimes also happens that one type may simulate another, e.g. *Cystoclonium purpurasceus* and *Agardhiella tenera*—*Batrachospermum fruticulosum* is a further example of the old parts of the thallus of one type (uniaxial) resembling the primary condition of the other (multiaxial), and in fact it can be described as pseudo-multiaxial. There is no essential difference between the transverse section of the old thallus (Pl. VIII, Fig. 11) and that of *Nemalion multifidum*, for example. The method of development of the two thalli is different, and while in both there is correlated growth of the axial strand, in *B. fruticulosum* the filaments are downwards growing and in *N. multifidum* they are upwards growing and organized into a definite growing-point. This similarity of old thalli, the one belonging to the uniaxial type and the other to the multiaxial, has been recognized by Kylin (1927, p. 93) and Fritsch (1945, p. 50), who states 'in fact several (*Cystoclonium*, *Catenella*) of the uniaxial forms are in the mature condition to all intents and purposes multiaxial'. While showing a fundamental correlation of the two types, this similarity does not indicate any phylogenetic relationship but does emphasize the need for studying the early stages of development, particularly where these changes take place early in the germling or very near the apex, and not as gradually as in *B. fruticulosum*. In this connexion it should be

stated that the first species of *Batrachospermum* to be examined showing this elaborated type of construction appeared to belong to the genus *Nemalionopsis*, created by Skuja (1934) for a species *N. Shawi* and described by him from an herbarium specimen from the Bataan Reserve on Luzon in the Philippines. However, longitudinal sections revealed remnants of the primary structure just above the node and further investigation showed it to belong to *Batrachospermum*.

Adventitious branches are known to occur fairly commonly throughout the Florideae, and Sirodot (1884) recognized their existence in *Batrachospermum*. The number of such branches, particularly on denuded axial strands, is the outstanding characteristic feature of their occurrence in *B. fruticulosum*. They develop both from the primary and secondary branches of limited growth as well as from the corticating filaments. After the axial strand has been denuded of its radial branches of limited growth their development is most abundant. This regenerative capacity of the axial strand is of undoubted biological value to the species in that new thalli can arise from the stumps of the previous season's growth. While not an established fact, it is possible that the plant may be reproduced by the production of such adventitious branches from fragments of an old thallus. This should be the subject of experimental tests, which the writer regrets it has not been possible to attempt. It also appears likely that *B. fruticulosum* would be a suitable subject for experimental work on the effect of varying conditions on the development of the corticating filaments as well as the factors influencing the change from the juvenile *Chantransia*-stage to the adult type of shoot.

While no special steps have been taken to ascertain the chromosome number of this species, it is worth recording that chromosomes have been counted in two nuclei in late prophase, one in a cell of an endophytic *Chantransia*-stage filament and one in a vegetative cell of a shoot of the adult type. In the former 22 chromosomes were present and in the latter between 20 and 22.

#### SUMMARY

A new species of *Batrachospermum*, *B. fruticulosum*, occurring in Derbyshire, England, is described. It belongs to the *Helminthoidea* section of the genus.

It is of particular interest morphologically as it shows a greater elaboration of the thallus than has been described previously for *Batrachospermum*. This is brought about by the activity of the filaments which corticate the central filament. In addition to giving rise to radially arranged branches of limited and unlimited growth, the corticating filaments produce a thick axial strand. This is the result of their number and their penetration of the primary central filament which they ultimately replace. The fully developed thallus may be described as pseudo-multiaxial.

This species is also characterized by a vigorous development of adventitious branches. These may originate from cells of the corticating filaments or from either the primary or secondary branches of limited growth. When denuded



of the radial branches of limited growth, the axial strand gives rise to these branches in large numbers.

In conclusion, I should like to express my thanks to Mr. E. Ashby for the photographs published as Pl. VIII. I am also greatly indebted to M. R. Lami, who as a result of careful comparison of specimens of *B. fruticosum* with specimens in the Thuret-Bornet and Chemin herbaria as well as with two series of collections of Sirodot's now in the herbarium of the Laboratoire de Cryptogamie in Paris, confirmed my belief that the plant in question is a new species of *Batrachospermum*. I also offer my thanks to Professor C. W. Wardlaw, in whose laboratory this work was carried out, and to Professor W. H. Semple for the Latin diagnosis.

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#### EXPLANATION OF PLATE VIII

Illustrating Dr. K. M. Drew's article, 'Anatomical Observations on a New Species of *Batrachospermum*'.

All figures are from untouched photographs. Figs. 5-11 are of different levels of the same axis.

Fig. 1. Fully grown plant collected June 21, 1942. Note the cylindrical basal parts of the thallus and the bushy growth. Photograph from fixed specimen. Nat. size.

Figs. 2 and 3. Young plants collected March 24, 1945. Photographs of living plants. Nat. size.

Fig. 4. Transverse section of very young axis. Whorls of branches of limited growth well developed but no cortication. ( $\times 80$ .)

Fig. 5. Transverse section of older axis. Cortication around central cell well developed and from these corticating filaments secondary branches of limited growth have arisen. Note difference in size between these and the primary branches. ( $\times 80$ .)

Fig. 6. Transverse section of still older axis. A few filaments have penetrated the lumen of the central cell and one of these in the process of penetrating through the wall is shown in Text-fig. 14. ( $\times 80$ .)

Fig. 7. Transverse section at a node, showing the base of the primary branches of limited growth as well as a rich growth of secondary branches of limited growth. Several filaments traversing the lumen of the central cell are shown in transverse section. ( $\times 80$ .)

Fig. 8. Transverse section showing the central cell completely full of invading filaments and surrounded by a thick layer of corticating filaments. ( $\times 80$ .)

Fig. 9. Central cell of section of Fig. 8 at a greater magnification. ( $\times 600$ .)

Fig. 10. Transverse section just above node, showing the remnants of the wall of the central cell and a clear area, the remnants of the lumen of the cell, traversed by a few invading filaments. ( $\times 80$ .)

Fig. 11. Transverse section of mature axis all trace of the original central cell having been lost. ( $\times 80$ .)

Fig. 12. Central cell into which several filaments have penetrated. The origin of the four-celled filament from a cell of the cortex is clearly shown. ( $\times 600$ .)

Fig. 13. Branch of unlimited growth arising near periphery of thallus from branch of limited growth. ( $\times 80$ .)

Fig. 14. Same branch at greater magnification. ( $\times 600$ .)

Fig. 15. Transverse section of old thallus worn back to central axis from which new growths are arising, including two branches of unlimited growth. ( $\times 80$ .)

Fig. 16. Very young branch of unlimited growth arising from similar thallus. Position indicated by arrow. ( $\times 80$ .)

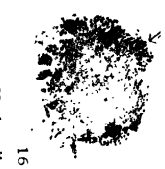
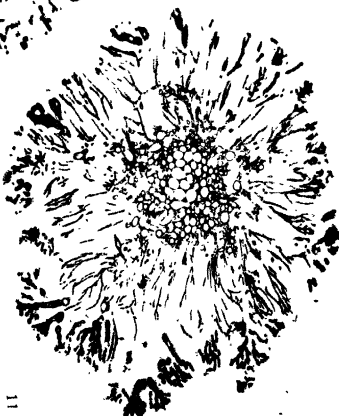
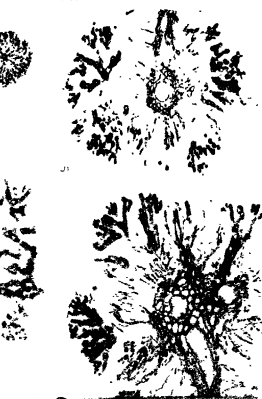
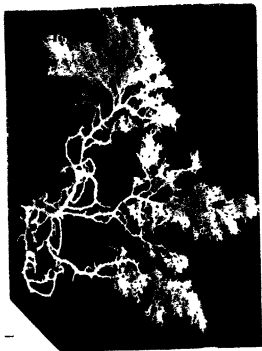
Fig. 17. Branch shown at greater magnification. ( $\times 600$ .)

Fig. 18. Two branches of unlimited growth arising from cell of corticating filament. ( $\times 600$ .)

Fig. 19. Transverse section of old thallus worn back to central axis. Rich growth of endophytic Chantransia-stage showing transition to adult type of axis. ( $\times 80$ .)

Fig. 20. Portion of filament of Chantransia-stage showing transition to adult axis. ( $\times 300$ .)





E. Ashby photo.

DREW - BATRACHOSPERMUM.

Hair cell.



# Studies in the Physiology of Leaf Growth

## III. The Influence of Roots on the Growth of Leaves and Stems in Rye

BY

R. S. DE ROPP

*(Research Officer of the Agricultural Research Council attached to Rothamsted  
Experimental Station)*

With two Figures in the Text

### INTRODUCTION

IN previous papers in this series comment has been made on the effect exerted by regenerated roots on the growth of isolated stem-tips of rye cultured on artificial media. It was observed that whenever the isolated stem-tip regenerated a root the apical meristem was stimulated into activity and further leaves developed whereas, in the absence of roots, growth was confined to the first leaf. This stimulating action exerted by the roots on the growth of the stem has previously been observed by Went (1938), who used pea seedlings as experimental material. He found that in pea plants deprived of their roots, stem growth was greatly reduced even when the cotyledons were left attached to the plant. He concluded from his experiments that 'a special substance is formed in the roots which moves upwards from cell to cell towards the apical parts on the pea stems where it causes growth in length in conjunction with auxin'. To this hypothetical substance Went gave the name of caulocaline.

The experiments to be described in this paper were designed to indicate the extent to which Went's findings applied to the growth of stems and leaves of rye. They comprised, (1) macroscopic and microscopic investigation of the stem with its regenerated root system, (2) a study of the effect of attached and isolated roots on the growth of isolated stem-tips, (3) a study of the purely absorptive role of the root and its effect on the growth of stem and leaf.

### MATERIALS AND METHODS

Both excised stems and roots were cultured on sucrose mineral agar by the method previously described (de Ropp, 1945). Material for histological examination was fixed in Bouin's solution and sectioned at  $8\mu$ . Special culture vessels (Fig. 1) made possible the study of the absorptive role of the roots. The side arm of the lower part of the vessel was filled with nutrient agar by means of a curved pipette. The scutellum of an excised embryo was placed in contact with the surface of the agar, and within a few hours adhered so firmly to it that no special device was necessary to hold the embryo in

place. The lower part of the vessel into which the roots grew was filled to the level of the side arm with distilled water or nutrient solution, introduced after the agar had set and before the embryo was placed in position. In some cultures the liquid in the lower part of the vessel was omitted, and instead the walls were lined with moistened filter-paper, so that the roots grew into

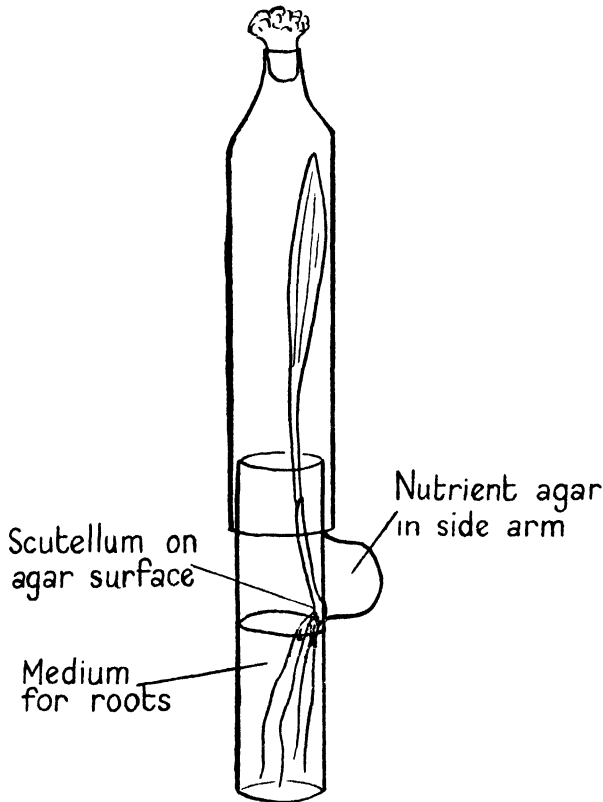


FIG. 1. Vessel for the culture of excised embryos enabling nutrients to be supplied through the scutellum or through the root.

a saturated atmosphere. In this way the effect of the absorption of water and of nutrients through the roots on the growth of the stem could be studied.

#### EXPERIMENTAL

##### *Structure of the isolated stem-tip with and without attached roots*

Fig. 2 shows the amount of growth made by an isolated stem-tip on which roots have been regenerated as compared with that made by a stem-tip devoid of roots. Both plants were cultured for 7 weeks in darkness on sucrose mineral agar containing 5  $\gamma$  of thiamin per c.c. In the plant without roots growth was confined entirely to the first leaf. The growing point of the stem and the

younger leaf primordia remained embryonic. In the other plant, where roots were regenerated, the growing-point of the stem showed renewed activity. The internodes elongated, 8 leaves emerged, and 2 adventitious roots developed at the nodes. There was also a progressive increase in leaf size correlated with the increased development of the root system. The smallness of the first leaf is explained by the fact that it had already reached its full size before the root system was regenerated and its basal meristem had become fully differentiated. The second leaf, though larger, also made very limited growth and was devoid of a leaf sheath, but the subsequent leaves all had leaf sheaths and the internodes became progressively longer at higher levels of the stem.

*Influence of the extent of the root system  
on the number and size of leaves*

Five groups of excised embryos were prepared in such a way that the stem-tip in each group had an increasing amount of embryonic root tissue attached to it. The groups were treated as follows:

Group 1. Isolated stem-tip, no root tissue attached.

Group 2. Stem-tip with one embryonic root primordium.

Group 3. Stem-tip with two embryonic root primordia.

Group 4. Stem-tip with complete embryonic root system but having scutellum and coleoptile excised.

Group 5. Complete excised embryo.

These five groups, each of which contained 20 embryos, were cultured on sucrose mineral agar for 2 weeks in darkness. At the end of this time they were removed and the following quantities determined: number of roots, number of emerged leaves, length of leaves, fresh and dry weight of tops and of roots. Means of all these values are given in Table I (p. 356).

Clearly from these figures there was a close positive correlation between the growth of the roots and that of the leaves. The length of the first leaf increased progressively from the first group to the last; from which it appears that the earlier the root system developed the more rapid was the growth of the first leaf. Rather surprisingly the greatest amount of total growth was made by those embryos from which the scutellum and coleoptile had been removed,

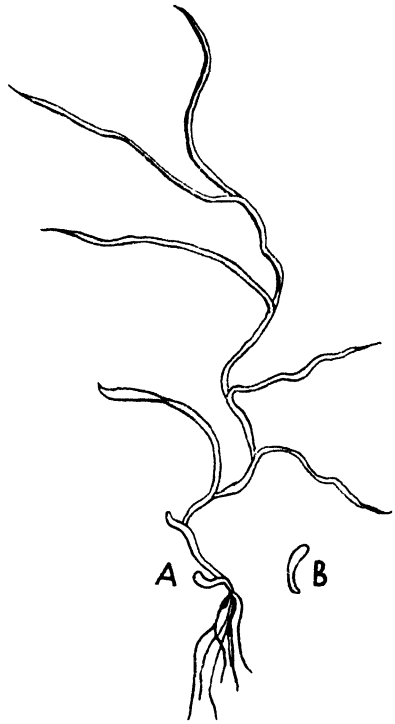


FIG. 2. Effect of regenerated roots on the growth of an isolated stem-tip of rye. A, stem-tip with roots; B, stem-tip without roots; both cultured in darkness for 7 weeks. ( $\times 2/3$ )



but the relationship between leaf growth and root growth still held, the greater weight of roots in group 3 being correlated with an increased weight of stem.

TABLE I

*Effect of the Amount of Root Tissue on the Growth of Leaves attached to isolated Embryos cultured for Two Weeks in Darkness on nutrient Agar. (Means of 20 estimations)*

Treatment.	Root no.	Leaf no.	Leaf length (mm.).				Weight of tops (mg.).		Weight of roots (mg.).	
			1st.	2nd	3rd.	4th.	Fresh.	Dry.	Fresh.	Dry.
No roots . . .	—	1'00	14.5	—	—	—	11.58	1'94	—	—
1 root . . .	1.23	1.69	17.5	32.6	54.2	—	22.06	3.23	3.15	0.30
2 roots . . .	2.58	2.33	20.7	43.4	64.0	70.0	55.53	6.46	14.30	1.92
All roots . . .	4.57	3.97	32.6	68.4	76.2	53.7	125.57	13.21	45.14	5.78
Whole embryo	3.94	2.70	89.8	104.3	67.4	—	88.82	9.00	27.82	3.06

(Standard deviations of these values were all about 10 per cent. of the means.)

### *Influence of detached roots on the growth of isolated stem-tips*

To determine whether any diffusible substance capable of affecting the growth of stems is produced by the roots, a group of 20 excised stem-tips was cultured on nutrient agar on which a root culture had already been established. These root cultures were prepared by excising aseptically the root-bearing portion of the rye embryo and placing it on the surface of sucrose mineral agar. Such excised fragments grew vigorously and produced a number of well-developed roots. After these root cultures had grown for a week the excised stem-tips were placed in direct contact with the cut surface at the top of the growing root system and the mixed cultures incubated in darkness for a further 2 weeks. As a control a group of 20 excised stem-tips were grown for 2 weeks on agar containing no roots. The amount of growth made by the excised stem-tips under these separate sets of conditions is shown in Table II.

TABLE II

*Effect of the Presence of a previously established Root Culture on the Growth of Isolated Stem-tips cultured in Darkness for Two Weeks on nutrient Agar. (Means of 20 estimations)*

Type of culture.	No. of emerged leaves.	Leaf length (mm.).	Weight of tops (mg.).	
			Fresh.	Dry.
Roots present . . .	1	17.82 ± 1.24	10.2 ± 1.00	1.7 ± 0.2
Roots absent . . .	1	16.78 ± 1.62	12.05 ± 1.93	2.5 ± 0.1

It is clear from these figures that the presence of the root cultures made no difference whatever to the growth of the isolated stem-tips.

### *Effect on leaf growth of absorption of nutrients by the roots*

To determine whether the effect of roots on stem and leaf growth was due entirely to the absorption of nutrients by the roots an experiment was set up in which the excised embryos were supplied with nutrients via the scutellum

only, the roots growing into a nutrient solution, distilled water or moist air. The special culture vessels previously described were used in this work, their side arms being filled with a 1 per cent. agar gel containing 2 per cent. sucrose and mineral salts. Three groups, each containing 20 intact embryos, were arranged. In group I the roots grew into a nutrient solution containing 2 per cent. sucrose and mineral salts. In group II the roots grew into distilled water and in group III into moist air. In addition two further groups were set up, group IV containing embryos with the scutellum intact and the roots excised, and group V consisting of excised stem-tips only.

All these cultures were incubated in darkness for one week, after which time the amount of growth of roots and leaves was estimated. None of the excised stem-tips in group V regenerated roots, but the embryos in group IV did so almost without exception. These roots were arranged to grow down into distilled water, making the group comparable in this respect with group II. Mean values for leaf and root growth are shown in Table III.

TABLE III

*Effect of the Uptake of Nutrients by Roots on the Growth of Leaves in excised Rye Embryos. Plants incubated for seven Days in Darkness. (Means of 20 estimations)*

Treatment	No. of emerged leaves.	Leaf length (mm.)	Weight of tops (mg.)		Root no.	Weight of roots (mg.).	
			Fresh	Dry.		Fresh.	Dry.
I Roots in nutrient solution	1	99.3 ± 5.1	71.3 ± 8.7	2.0 ± 0.1	3.0	15.1 ± 1.4	1.4 ± 0.1
II Roots in distilled water	1	80.8 ± 4.0	23.1 ± 2.1	1.7 ± 0.1	3.4	15.6 ± 1.3	1.5 ± 0.1
III Roots in moist air	1	57.9 ± 2.9	15.1 ± 1.7	1.3 ± 0.1	3.5	14.9 ± 1.6	1.8 ± 0.1
IV. Scutellum attached	1	42.4 ± 2.1	9.3 ± 1.0	1.0 ± 0.1	2.3	4.9 ± 0.5	0.7
V Stem-tip only	1	17.1 ± 0.9	5.6 ± 0.5	1.0 ± 0.1	—	—	—

It is evident from these figures that the effect of the roots on the growth of the leaves was only to a small extent due to the uptake of nutrients by the roots. This is made clear by a comparison of the values for leaf growth in groups I and II. In group III the reduction in length and fresh weight of the leaf evidently resulted from the inability of the root to absorb sufficient water. In group IV, which is comparable with group II, the reduced size of the root system was reflected in the greatly reduced amount of growth of the tops. In general it will be observed that the absence of roots affected leaf length and the fresh weight of tops to a far greater extent than it affected the dry weight of the tops.

#### DISCUSSION

The foregoing experiments indicate that the roots exert a stimulating action on the growth of the stem and leaf in rye. This action does not seem to depend entirely on the absorptive function of the root and may be attributed to some physical or chemical influence which the root is able to exert only when it is in direct organic union with the stem. These findings accord very closely with those recorded by Went (1938), working with peas. He also found that the effect of the root on the stem growth of the pea cannot be entirely attributed

to the uptake of nutrients by the root. Furthermore he showed that no growth-promoting effect is transferred from an isolated root system to a stem placed on its cut surface unless, through the union of the parts, tissue continuity is established.

As Went has already observed, the work of Hagemann (1932) on leaf cuttings lends support to the view that the roots exert a stimulating action on the growth of the stem. This worker investigated the regenerative capacity of leaves from 602 species of plants selected from 82 families. Out of this total the leaves of 213 species regenerated roots alone, 99 species regenerated both shoots and roots, and only 8 species, confined to 2 families, produced shoots without roots. Amongst the plants which produced both shoots and roots it was found that the shoots were invariably produced after the roots.

Nevertheless, the work of Shih-Wei Loo (1945) has shown that shoots are capable of continuing to grow without an attached root system. This worker maintained isolated stems of asparagus through as many as twenty transfers on an artificial medium, and concluded that the growth of such stems under the conditions he employed was potentially unlimited. He states, however, that stems which regenerated roots grew 4 or 5 times as rapidly as did those which failed to regenerate roots. He suggests that two groups of factors or substances promote the growth of isolated asparagus stems, one produced in the stem in the presence of light but not in darkness, the other generated in the roots and transferred from them to the stem. Loo's hypothetical light-generated factor does not appear to be necessary for the growth of stems and leaves of rye. Provided roots were regenerated it was found that stems and leaves would make considerable growth in continuous darkness. But the effect of roots on stem growth was unquestionable. Whether this effect was due to the production of a specific hormone, 'caulocaline', acting on the distribution of auxin as postulated by Went cannot be finally decided without further investigation.

#### SUMMARY

Experiments were performed to test the effect of roots on the growth of the stem and leaf in rye.

It was shown that, in isolated stem-tips which regenerated roots, continued growth of stems and leaves occurred, whereas in stems which failed to regenerate roots growth was confined entirely to the first leaf of the stem.

The amount of stem and leaf growth was shown to increase with increase in the size of the attached root system.

The effect of the root system on the growth of the stem was found to be attributable only partially to the absorptive action of the root.

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Physiological and Ecological Studies in the Analysis of  
Plant Environment

1. The Light Factor and the Distribution of the Bluebell  
(*Scilla non-scripta*) in Woodland Communities<sup>1</sup>

BY

G. E. BLACKMAN

(Department of Agriculture, Oxford)<sup>2</sup>

AND

A. J. RUTTER

(Department of Botany, Imperial College of Science and Technology, London)

With nine Figures in the Text

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INTRODUCTION

DURING the last few decades the results of investigations in several botanical fields have all contributed to a better understanding of the general principles which determine the type of plant community under any well-defined but broad range of environmental conditions. The importance of climate in governing the nature of the vegetation has been established without question while the floristic composition of typical communities in various parts of the world have been observed by numerous workers. In spite, however, of the very considerable advances made, precise information on the relative importance of the environmental factors for any one community are lacking. The principal factors likely to be operative under any given set of conditions can be assessed, but where several factors are seemingly involved

<sup>1</sup> Some of the results included in this paper were presented by the second author in a thesis for a Ph.D. degree of the University of London.

<sup>2</sup> Formerly of Imperial College of Science and Technology.

the role of each factor or the interaction between factors has not in general been precisely determined for the individual species characteristic of the community.

The present series of investigations is an attempt to examine critically the complex of factors responsible for the distribution of an individual species by coupling autecological studies with more controlled experiments in the field and the laboratory. This and subsequent papers are concerned with the factors affecting the distribution of the ground flora in woodland, and in particular the autecology of the bluebell, *Scilla non-scripta*.

In the past much attention has been given to distinguishing the different woodland communities, their floristic composition, and the broad environmental, soil, and climatic factors governing such differentiation. Other papers have been concerned with specific problems such as regeneration, and these more detailed studies have served as a basis for formulating general conclusions as to the main environmental factors governing the distribution of the ground flora. Nevertheless, though the data are abundant, many of them on modern statistical standards lack precision, and there is an absence of accurate and comprehensive information on the autecology of any individual woodland plant.

It is quite evident that variations in light intensity have a marked effect on the distribution of species in woodland, and one of the first accounts to stress the importance of the light factor was given by Salisbury (1916-18), who investigated the ground flora of the oak-hornbeam woods of Hertfordshire. In one woodland site measurements of light intensity were made at fairly frequent intervals with photographic paper. Since the absorption by the leaves of the red end of the spectrum is greater than that of the blue-violet end, to which photographic paper is sensitive, the method has the drawback of underestimating the light intensity in woodland, but it was nevertheless the only one available at the time of the investigation. Using this technique, it was found that the initial value of about 0.60 daylight at the end of March fell to less than 0.05 daylight by the middle of May. It was observed that plants such as *Ficaria verna*, *Mercurialis perennis*, and *Primula acaulis*, which develop their leaves by the middle of January, were in summer to be found in deeper shade than other plants whose leaves were not produced until March, such as *Galeobdolon luteum*, *Conopodium denudatum*, and *Anemone nemorosa*. Salisbury therefore concluded that these plants which produced the earliest foliage were favourably placed to survive deep shade in summer, owing to active photosynthesis during the high light phase prevailing at the beginning of spring.

Daxer (1934) carried out quadrat investigations on the ground-flora communities of both deciduous and evergreen woods. The dry weight per quadrat of the total ground flora rose steeply with increasing light intensity, and since the slope of the curve showed no falling off at the edge of the wood, it appeared that even at these high light intensities light was a limiting factor. Individual species within the communities differed in their reaction to light. Some showed the same trends as the dry weight of the total ground flora, while

others, e.g. *Oxalis acetosella* and *Geranium robertianum*, gave decreased dry weights per quadrat as the edge of the wood was approached. These divergencies cannot be explained on the simple assumption made by Daxer. It is clearly the result of a series of complex interactions involving the varying density of a number of species and their individual reactions to light intensity, water-supply, and nutrient level.

Filzer (1939) also carried out quadrat investigations in a coniferous wood and obtained similar results. He postulated, on somewhat slender evidence, that the physiological relationship between dry weight production and light intensity is linear within the range of 0.72 and full daylight, and he attributed the falling away, in natural habitats, from this linear relationship at the higher light intensities to 'root' competition, i.e. presumably competition for water or mineral nutrients or both.

Considerable attention has been given by American workers to the question of 'tolerance', that is to say the ability of trees to grow in close stands, and the readiness with which seedlings establish themselves in the shade of the parent tree. Several attempts, of which the most frequently cited is that of Zon and Graves (1911), have been made to classify trees on the basis of their degree of tolerance. Three to five tolerance groups have usually been recognized, and their differentiation is based on a dozen or more observational criteria such as the density of the crown, the rapidity both of self-pruning by individual trees and of self-thinning by the stand, together with the general condition of the saplings occurring beneath the older trees.

It was assumed by early workers that differences in 'tolerance' were chiefly due to the different light requirements of species, but it has since been recognized that competition for water and soil nutrients may be equally important as competition for light in determining the ability of seedlings and saplings to grow beneath their parent stands. This question has been fully discussed by Toumey (1928), Pearson (1930), and Baker (1934).

Few attempts have so far been made to obtain quantitative expressions of the relations between light and growth, or to grow trees in artificial shade and under controlled conditions. Shirley (1932) recorded the numbers of saplings and seedlings of three pine species, their annual height growth, and the light intensity on 50 sites in virgin forest of Norway pine (*Pinus resinosa*). The light intensity was measured with a thermopile at 10 points on each site, but only once during the summer in which the experiment was conducted. The number of young trees per acre was found to increase with light intensity up to full daylight. Establishment of *P. resinosa* seedlings was uncertain below 0.17 daylight, and where shrubs occurred and reduced the light to less than 0.05 daylight, coniferous seedlings were absent. The optimum light intensity for annual height growth in *P. resinosa* was 0.63 daylight, for White pine (*P. strobus*) 0.36 daylight, and for Jack pine (*P. banksiana*) 0.75 daylight.

Holch (1931), investigating five tree species in three communities, with a varying light intensity of 1.00, 0.104, and 0.035 daylight, according to the



type of vegetation, concluded that seedling growth of both root and shoot, irrespective of species, was greatest in daylight and least in 0.035 of daylight. This type of experiment can be strongly criticized on the grounds that the three sites differed in many respects other than light intensity, for they are described as prairie, bur oak forest (*Quercus macrocarpa*), and linden forest (*Tilia americana*). Height, number of leaves, leaf area, and similar quantities were used to characterize growth, the only one involving weight being the increase in dry weight of unit leaf area.

Seedlings of *P. ponderosus* were grown by Pearson (1936) for 5 years under replicated lath screens which gave light intensities of approximately 1.00, 0.50, 0.20, and 0.10 daylight. At the two lower light levels only a few plants survived, whereas at 0.5 daylight good growth was made, though the plants were shorter and the main stems half as thick as those of plants receiving full light.

From the foregoing account it is evident that over a wide range of woodland conditions and in many different communities light intensity must play a considerable part in governing both the distribution of the ground flora and the regeneration of the constituent trees. Nevertheless the available data at the same time serve to emphasize the need for more quantitative information. For no single species, even in one woodland community, has the importance of the light factor yet been accurately assessed.

This lack of critical data can largely be attributed to the difficulty of planning ecological investigations in which the assessment of how the light factor operates can be determined with precision. It is only in recent years that statistical methods of experimentation and analysis of the data have made it possible to differentiate the complex of factors concerned in most biological problems. It was with the appreciation that these statistical techniques would be of great assistance that the present investigations were undertaken.

On *a priori* grounds it seemed that the method of multiple regression would be of most value in correlating the light factor with changes in plant density. As it appeared more than probable that light intensity was the most important factor, Dr. Bartlett suggested that the importance of all other factors could be tested by utilizing his technique of a pseudo-variate.

In order that the methods could be used to the full advantage it was essential that the species investigated should be one in which individual plants could be readily distinguished and counted. From this aspect the bluebell had much to recommend it since individual plants are well-defined entities. From other aspects it seemed equally desirable. The bluebell is a frequent component of the ground flora of English woodland, and it is easy to collect large quantities of bulbs in order to carry out parallel investigations under more controlled conditions. It is also well suited for transplant experiments or for investigations of growth and development, since it can be lifted, weighed, and replanted in the dormant phase. Moreover, its use would throw light on the general ecology and physiology of a bulbous monocotyledonous plant.

Finally, although *S. non-scripta* may be regarded as a plant of woodland communities, there are also places where it occurs in bracken and grassland.

In some of these open situations it is assumed to be a survival from woodland felled several decades previously, but this is not sufficient to explain its occurrence in other similar localities.

Since growth in the open is possible, the more common restriction of *S. non-scripta* to woodland does not appear at first sight to be associated with intolerance of high light intensity. The causes underlying the varying distribution of *S. non-scripta* was one of the problems faced at the outset of these investigations, and its elucidation clearly required controlled experiments on other factors likely to have an adverse effect on the establishment and growth of plants in open situations. This first paper is concerned with the light factor in woodland, other aspects of the autecology and physiology of the bluebell will be dealt with subsequently.

### EXPERIMENTAL METHODS

#### 1. *Measurement of light intensity*

The method employed to measure the light intensity was, with a few modifications, that described by Atkins, Poole, and Stanbury (1937). The measurements were made with two Weston rectifier cells, in which the sensitivity to varying wave-lengths approximates to that of the human eye. Some workers have made measurements of light intensity on a plane normal to the sun's rays, but for two-thirds of the hours of daylight in England the sky is overcast. Moreover, when there are clouds of the cumulus type maximum intensity may not come from that quarter of the sky in which the sun is situated; particularly in woodland, the light from the sky accounts for the greater part of the total radiation. Atkins and others consider that even when 25 per cent. of the floor of a wood is covered by sunflecks when the sun is high, only about 5 per cent. of the total radiation falling on any point is due to direct sunlight. Consequently throughout this series of experiments the cells were placed on a horizontal plane, and were covered with diffusers of opalescent glass in order to integrate light coming from all parts of the sky. The cells were connected by low-resistance cable through a two-way switch to an ammeter, so calibrated as to give direct readings in foot-candles. One cell stood in the open, away from all shade, and the other was placed at the point whose light intensity, relative to full daylight, was to be determined. By means of the two-way switch, almost simultaneous comparative readings from the two cells were obtained. As, however, the two cells were not completely 'matched', a correction factor was obtained by placing them side by side in full daylight and taking ten comparative sets of readings. Such a correction factor was determined whenever a series of light intensities was estimated, since it was thought that the factor might vary slightly with temperature changes.

Atkins, Poole, and Stanbury demonstrated the relative unimportance of sunflecks compared with diffuse light, but a serious error in measurement is caused when a sunfleck falls on the cell within the wood. To eliminate this error, measurements were made only when the sun was obscured by cloud and there were no shadows detectable by the eye. As a further means of

standardizing the method of measurement, observations were made within about 2 hours of midday so that the ratio of the vertical component to the total radiation was more or less constant.

## 2. Design of experiments

The plan of the experiments was in part dependent on the site investigated. The area that could be selected in any woodland community was limited by the need to have one of the photo-electric cells outside the wood in an open

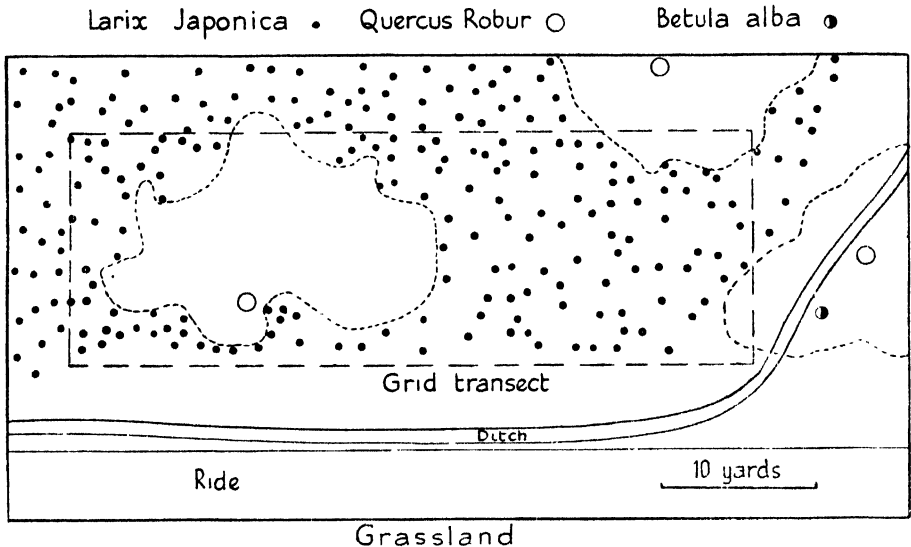


FIG. 1. Experiment I, Larch-oak wood. Topography of site showing position of grid transect, the distribution of larches, and the spread of the oak-tree canopies.

situation. But since at the periphery there is in general a more pronounced light gradient than deeper in the wood, the choice of site was not so narrow as would first appear.

Having selected a suitable area, a series of parallel transects at right angles to the edge of the wood were laid down. On these transects other transects parallel to the wood edge were superimposed, making a grid with 200–400 points. At each intersection of the grid the density of the bluebells was obtained by counting the number of plants in a quadrat—the size of the quadrat being dependent on the density. Similarly the degree of shade was estimated for each of the points where density estimates were made.

## EXPERIMENTAL RESULTS

### 1. The relationship between light intensity and bluebell density

(i) *The light factor and the distribution in a larch-oak woodland.* The site chosen for the first experiment was at Shurlock Row in Berkshire on the edge of the Bagshot Sand. The area had in the past been woodland which had

been replanted with Japanese larch some thirty years previously. The trees had not been finally thinned and were overcrowded and spindly. In consequence all but the uppermost branches had died back and there was much brushwood on the ground. In the area investigated there was a single large

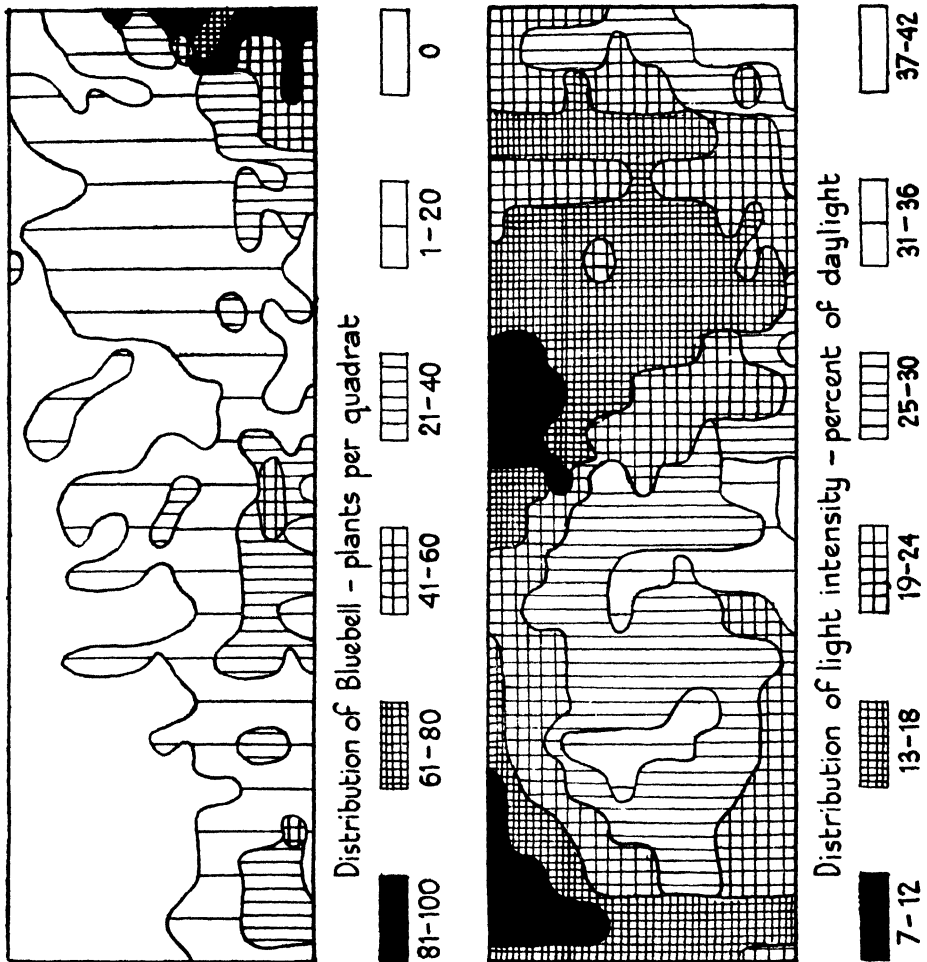


FIG. 2. Experiment I, Larch-oak wood. Patterns of the distribution of bluebell density (plants per 9 in.  $\times$  9 in. quadrat) and the degree of shading at 'ground' level within the grid transect.

oak tree while the canopy of two other oak trees extended over the area. Under the oak trees there were no larch trees and the ground flora consisted entirely of *S. non-scripta*. As the bluebell density was greatest at the extreme edge of the wood, which abutted on a wide cart-track with open scrub beyond, the base transect was laid at the wood edge parallel to the track. Along this transect at 3-ft. intervals 23 transects, each 45 ft. long, were run at right angles into the wood. Again at 3-ft. intervals along each of these transects,

the density was estimated by counting the numbers of bluebell plants in a 9-in. quadrat, making a total of 345 observations. Similarly the light intensity was measured at each of these points on May 5, 1937, at a time when the oak leaves were not fully expanded. In addition the position of each larch tree was mapped. The topography of the area, together with the gradient in light intensity and density of *S. non-scripta*, are shown in Figs. 1 and 2. The 'contour' lines for density indicate that the bluebells are more abundant near

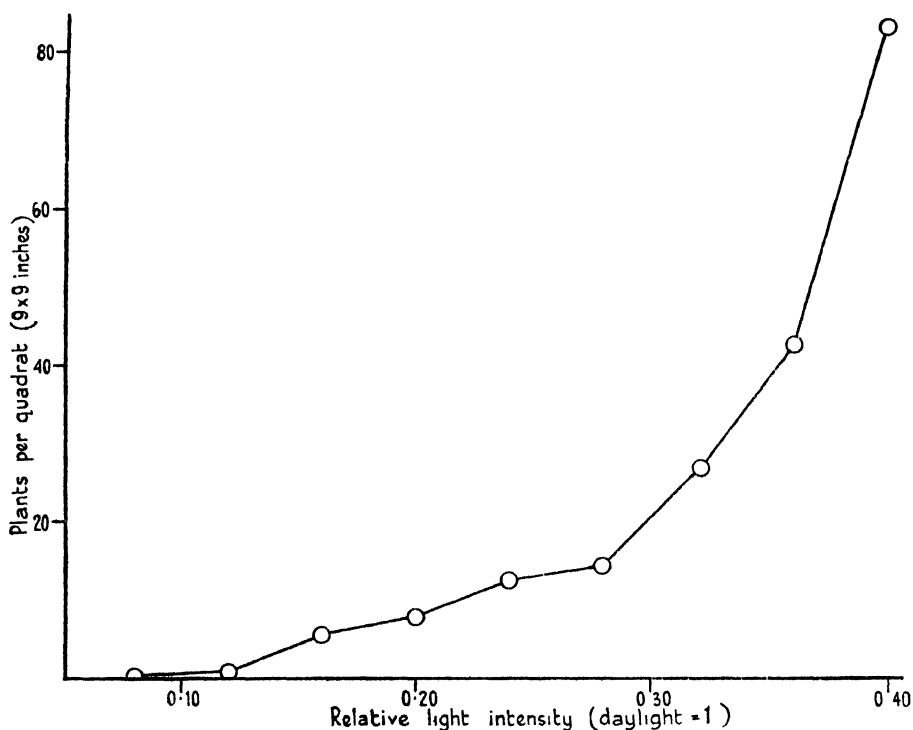


FIG. 3. Experiment I, Larch-oak wood. The relationship between bluebell density and the degree of shading at 'ground' level.

the wood edge. Comparison with the light contours reveals that the bluebells are densest where the intensity exceeds 0.24 of daylight and are rare below an intensity of 0.12.

Since Fig. 2 points to some interdependence between the distribution of *S. non-scripta* and light intensity, this interrelationship can be further analysed by splitting up the light data into a number of equal classes and calculating from the corresponding density data the mean density figure for each light class. These results are shown in Fig. 3 and bring out clearly the marked relationship between density and light intensity on this site.

Inspection of Fig. 1 shows that the site was far from uniform owing to the presence of the oaks. Thus if there was a difference between the oak and larch effects on the bluebell, other than the different degree of shading, then

this effect can only be readily separated from the light effect by statistical analysis of the data. For this purpose the method of regressions was used. In the analysis the possible effect of a difference between oak and larch trees was represented by a pseudo-variate  $z$ . To each point at which records had been made, a value of 1 or 0 was assigned according to whether the point was, or was not, beneath the canopy of an oak.

The area was divided up into 25 blocks, 20 of which contained  $5 \times 3 = 15$  points, and the remaining five  $3 \times 3 = 9$  points. The terms used in the calculation of the regression were:  $y$  = square root of number of plants per quadrat;  $z$  = tree factor as above;  $x$  = light intensity as a fraction of daylight (full daylight = 1).

The square root of plant numbers was used, since with whole numbers the distribution is discontinuous. The use of the square root transformation results in a more nearly normal distribution of the variate.

The variation of the data was divided into two sources, namely, between blocks with 24 degrees of freedom and within blocks with 320 degrees of freedom.

For each of these two sources the sums of squares and variance appropriate to the total and partial regressions of the light factor ( $x$ ) and the tree factor ( $z$ ) on the square root of the plant density ( $y$ ) were found and compared with the remaining variance (Table I).

TABLE I

*Experiment 1. The Distribution of S. non-scripta in a Larch-Oak Wood. Analysis of Variance of Regressions of the Square Root of Plant Density ( $y$ ) on Light Intensity ( $x$ ) and the Tree Factor ( $z$ ) within and between Blocks*

		Degrees of freedom.	Sum of squares.	Variance.	F.	F required.	
						5 per cent.	1 per cent.
Between blocks	Regr. on $x$	1	448.4	448.4	16.9	4.30	7.94
	Partial regr. on $z$	1	286.4	286.4	10.8	—	—
	Regr. on $z$	1	0.0	0.0	—	—	—
	Partial regr. on $x$	1	734.8	734.8	27.6	—	—
	Multiple regr. on $x$ and $z$	2	734.8	367.4	13.8	3.44	5.72
	Remainder	22	585.7	26.6	—	—	—
Total between blocks		24	1,320.5	—	—	—	—
Within blocks	Regr. on $x$	1	36.9	36.9	15.9	3.87	6.72
	Partial regr. on $z$	1	7.4	7.4	3.19	—	—
	Regr. on $z$	1	12.6	12.6	5.43	—	—
	Partial regr. on $x$	1	31.7	31.7	13.7	—	—
	Multiple regr. on $x$ and $z$	2	44.3	22.2	9.56	3.03	4.68
	Remainder	318	738.7	2.32	—	—	—
Total within blocks		320	820.0	—	—	—	—
Grand Total		344	2,140.5	—	—	—	—

From this analysis of variance it is clear not only that the blocks account for about 60 per cent. of the total variation, but also that any regression of density on light intensity and the tree factor depends almost entirely on the variations between blocks (734.8 compared with 44.3).

Before setting out the regression coefficients calculated from the block differences, however, the result of a further division of the 25 blocks into 5 rows and 5 columns was considered. The following analysis of variance (Table II) shows that any block relations of density with light and the tree factor are accounted for almost entirely by row and column effects, the remaining regression terms being less than error.

TABLE II

*Experiment 1. The Distribution of S. non-scripta in a Larch-Oak Wood. Analysis of Variance of Regressions of the Square Root of Plant Density ( $y$ ) on Light Intensity ( $x$ ) and the Tree Factor ( $z$ ) after grouping blocks in rows and columns*

		Degrees of freedom.	Sum of squares.	Variance.
Rows	Regr. on $x$	1	637.7	637.7
	Regr. on $z$	1	22.0	22.0
	Multiple regr. on $x$ and $z$	2	706.3	353.2
	Remainder	2	65.0	32.5
	Total between rows	4	771.3	—
Columns	Regr. on $x$	1	79.8	79.8
	Regr. on $z$	1	5.3	5.3
	Multiple regr. on $x$ and $z$	2	337.6	168.8
	Remainder	2	31.8	15.9
	Total between columns	4	369.4	—
Remaining block variation.	Regr. on $x$	1	2.0	2.0
	Regr. on $z$	1	0.0	0.0
	Multiple regr. on $x$ and $z$	2	2.7	1.4
	Remainder	14	177.2	12.7
	Total remaining block variation	16	179.9	—
	Total between blocks	24	1,320.6	—

In considering the coincidence of the variation with positional effects, it should be borne in mind that if the site had been evenly covered with larches of uniform size, then a column effect of light intensity would be expected. The contribution of the lateral light from the open edge to the total light received on the floor of the wood would decrease as the distance from the edge of the wood increased. On the other hand, there should be no row effect, since the light gradient due to the lateral light should be at right angles to the edge of the wood and parallel to the column transects.

From Figs. 1 and 2 it is evident that, due to the presence of the oak trees and the irregular distribution of the larches, the light contours do not run

parallel to the wood edge. Thus a row effect would be expected in the analysis. Nevertheless, in view of the high proportion of the variance accounted for by row effect, the observed significance of plant density on light intensity and the tree factor (Table I) does not necessarily imply any causal relationship. From the ecological viewpoint there is no likely alternative explanation other than that light has a controlling influence on the density of *S. non-scripta*. It can, however, be argued that the correlation between density and the tree effect may be controlled by a third factor and is not a direct effect of the tree species on density.

From the data it is also possible to calculate a multiple regression relating the square root of bluebell density ( $y$ ) with the intensity ( $x$ ) and the tree factor ( $z$ ), viz.

$$y = 35.11x - 3.33z - 4.26.$$

This equation summarizes the *whole* of the information available from the 345 points. From it the conclusion is reached that, for a given light intensity, the population of bluebells beneath oak trees is less dense than under larch trees. It can further be calculated that the average difference in density between the oak and the larch stations is almost equally contributed to by differences in light intensity and the tree factor. Since the mean light intensity under larches is 0.187 daylight and under the oaks 0.267, then from the equation it follows that the mean density difference is 0.52.

Since  $y$  is expressed in terms of the square root of density, then the square of 0.52, viz. 0.27, represents the mean difference in density between the two types of situation, a negligible quantity compared with the range in density observed in the quadrats, namely, 0–80 plants. Hence, between the oak and larch stations the positive effect of increased light intensity is offset by the negative effect of the tree factor.

On this basis it is, therefore, clear why in Table I the simple regression on the tree factor ( $z$ ) is negligible, while the partial regression on  $z$  is highly significant.

In addition to the multiple regression, a simple regression of the square root of density ( $y$ ) on light intensity ( $x$ ) can also be calculated and has been found to be:

$$y = 21.33x - 2.40 \quad (\text{standard error of } b = 4.29).$$

From this equation, by substituting the value of nought for density the light intensity can be calculated at which bluebells fail to establish themselves. Under the given conditions for this woodland community, where the intensity in early May is less than 0.11 of daylight, no plants are found. This light value can be termed the 'extinction point' and it should be stressed that this value has been arrived at by taking into account *all* the data and is not confined to a consideration of the data for the quadrats in which bluebells were not found.

(ii) *The light factor and the distribution in a beech wood.* The site for this experiment was a mature beech wood on the Berkshire Downs near Yattendon. The wood was of the plateau type with a slight downward slope facing north



and the chalk was some 12–18 in. below the surface. The area chosen for the experiment was close to the south edge of the wood and a base transect was laid out parallel to the edge (see Fig. 4). As in the oak-larch wood, 20 transects at right angles to the base were measured off at yard intervals and observations were made at 10 points, a yard apart, along each transect. Thus the density measurements and the light determinations were carried out on a grid of  $10 \times 20$  points, i.e. 200 in all. For the density estimate, the number of bluebells

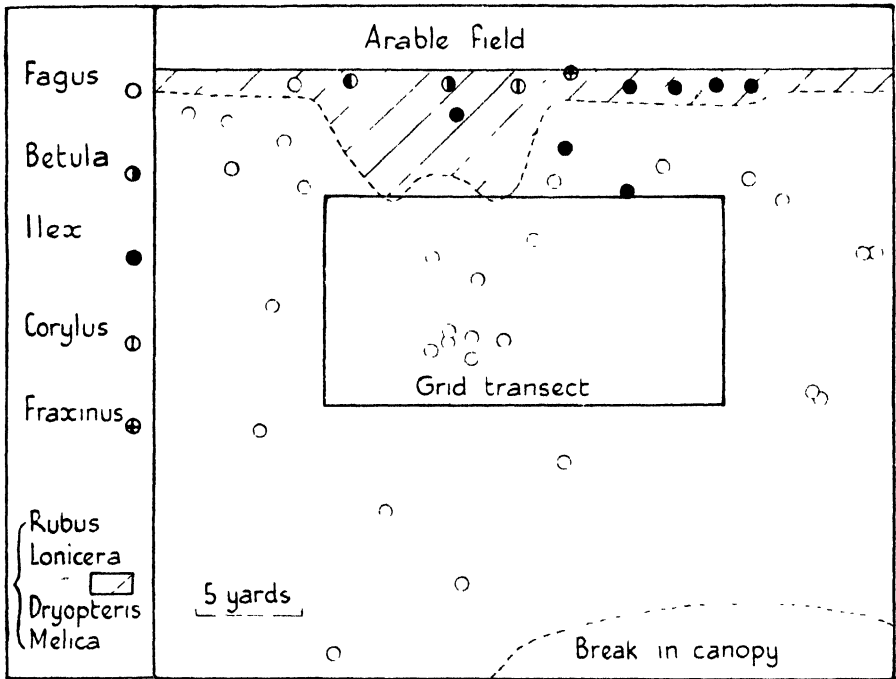
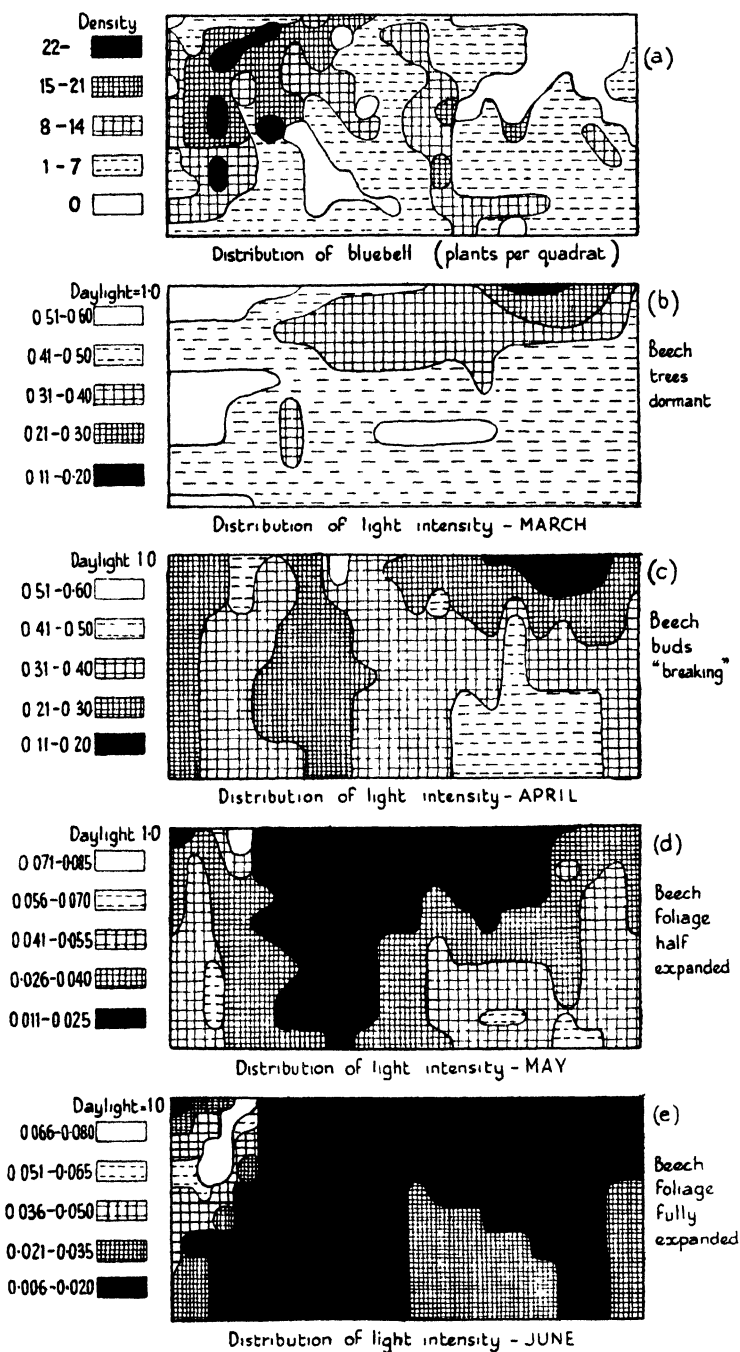


FIG. 4. Experiment 11, Beech wood. Topography of site showing position of grid transect, and the distribution of beech and other species.

was counted in a square quadrat, 9 in. across. The light measurements were made, as before, by comparing the current generated by two photo-electric cells, one within the wood and one in the open.

The variation in the density of *S. non-scripta* is shown in Fig. 5 *a*. On the right half it is seen that the density is least close to the edge of the wood, whereas on the left the position is more complicated. There is towards the middle an area of low density which shows no positional effect of depth, but on the left the density increases rapidly especially towards the wood periphery. When the site was first examined this distribution immediately struck the eye and seemed likely to be associated with the variation in lateral light reaching the floor of the wood. Although the experimental area was wholly within the canopy of the beech trees, along the wood edge a number of other trees had established themselves—hollies on the right and hazel, birch, and ash on the



FIGS. 5 a-e. Experiment II, Beech wood. Fig. 5 a. Pattern of the distribution of bluebell density (plants per  $9 \times 9$  in. quadrat) within grid transect. Figs. 5 b-e. Changes in the pattern of light intensity with the seasonal increase in shading due to leaf expansion of the overhead canopy.

left (see Fig. 4). Thus in the early spring, before the beech leaves had expanded, lateral light would be more effectively blanked off by the hollies than by the other deciduous species. Moreover, on the right there was a break in the canopy, and this was opposite the locus of the highest bluebell density on this side. Finally the distribution of the ground flora was associated with the differences in the peripheral trees. Under the hollies on the woodland side the ground was bare, but under the ash, birch, and hazel, there was a mixed stand of *Rubus*, *Lonicera*, *Melica uniflora*, and *Aspidium filix-mas*. On the other hand, within the experimental area *S. non-scripta* was by far the commonest species; other species consisted of young seedlings of ash and small plants of *Viola sylvatica*, both of which were rare.

As other experiments had shown that *S. non-scripta* begins active growth in early April before the normal expansion of the beech leaves, it was evident that in correlating the distribution of the bluebell with light intensity a single determination of intensity was not likely to give the most information. Accordingly the light was measured on four occasions, namely:

- March 29. Beech trees dormant; bluebell leaves newly emerged.
- April 24. Beech leaves expanding; bluebell leaves nearly fully expanded.
- May 18. Beech leaves fully expanded; bluebells in flower.
- June 26. Beech leaves mature and dark green; bluebell leaves dying.

The distribution of the light intensity within the experimental area on the four occasions is seen in Figs. 5 *b-e*. In March the effect of the holly trees in decreasing the lateral light is evident, since the light gradient increases away from the wood edge. On the right where the light intensity is higher, due to the break in the canopy, there is no well-defined gradient. By April, behind the hollies, the rising light gradient away from the edge is still evident, but on the left the distribution of light is radically altered, a strip of low intensity lying each side of an area of relatively high light opposite the gap in the peripheral brushwood. Again in May the effect of the gap in maintaining a relatively high light is evident, and there is still on the right a marked increase in intensity away from the edge, to which the break in the beech canopy to the north (Fig. 4) contributes. Between these two areas there is a zone where there is relatively uniform and pronounced shading. This zone is even more marked in June and divides the still evident gradient on the left from the localized area of high intensity near the break in the canopy.

A comparison of the four light gradients with the bluebell density distribution in Fig. 5 suggests that the initial assumption of a relationship between the bluebell density and seasonal light intensity was correct.

This assumption can be further and more accurately tested by calculating the multiple regression correlating the density with the light intensities on the four occasions. Since one-fourth of the quadrats contained no bluebell plants, and this high proportion of noughts made the data unsuitable for the calculation of regression terms, the 200 points were divided into blocks of 4 points each; the numbers of plants for the group of the four quadrats were added

together and the mean light intensity on each of the four occasions calculated. In this way, in only two blocks was the light intensity represented by nought. Using the regrouped data the following regression was obtained:

$$y = 10.4x_1 + 17.0x_2 - 109.6x_3 + 102.9x_4 - 3.99,$$

where  $y$  equals the square root of the plant density and  $x_1$  to  $x_4$  are respectively the light intensities relative to daylight in March, April, May, and June. That the regression is highly significant can be seen in Table III. It should also be noted that half the variation of the bluebell density can be accounted for in terms of the light intensity.

TABLE III

*Experiment II. Distribution of S. non-scripta in a Beech Wood. Analysis of Variance due to the Multiple Regression of Plant Density on Light Intensity at Four Successive Occasions*

	Degrees of freedom.	Sum of squares.	Variance.	F.
Multiple regr.	4	112.98	28.25	10.99 ( $P = 0.01$ level)
Error	45	115.68	2.57	—
Total	49	228.66	—	—

Each of the four partial regression coefficients given in the equation is significant, the values of ' $t$ ' obtained for  $b_1$ — $b_4$  being 2.293, 2.937, 4.004, and 3.705 respectively, whereas the required value of  $t$  at the significant level of  $P = 0.05$  is 2.014.

It is seen that  $b_1$  and  $b_2$  are less than  $b_3$  and  $b_4$ ; this implies that, at the levels of light intensity studied, a change in light intensity has a greater effect on density in May and June than it does in March and April. In effect, however, the intensity on the first two occasions is most important in determining density, since the general light level is then higher. This is illustrated by multiplying the mean values of  $x$  by their corresponding partial regression coefficients:

$$\begin{array}{ll} b_1 \bar{x}_1 = + 4.57 & b_2 \bar{x}_2 = + 5.39 \\ b_3 \bar{x}_3 = - 3.43 & b_4 \bar{x}_4 = + 2.02. \end{array}$$

From the negative value of  $b_3$  it must be assumed that when the effect of other occasions is eliminated, high light may have an adverse effect on the bluebells; a result for which it is difficult to offer any explanation.

In addition to the multiple regression for all light occasions, regression coefficients of the square root of plant density ( $y$ ) on light intensity ( $x$ ) were calculated for each of the four separate occasions from the block terms used to obtain the multiple regression. All the values were positive, but the coefficient of  $x_3$  was not significant. A summary of the data for each occasion is given in Table IV.

In each regression by substituting nought for the square root of plant density the level of light intensity below which bluebells would no longer be expected (extinction point) can be obtained for each date. The extrapolation of the regression for June 26 gives the result that even if the light intensity

TABLE IV  
*Experiment II. The Relationship between Density of S. non-scripta and Light Intensity on Four Occasions in a Beech Wood (Light Intensity Data expressed in Terms of Full Daylight)*

March 29				April 24				May 18				June 26			
No. of blocks.	Light intensity.	Plants per 4 quadrats.		No. of blocks.	Light intensity.	Plants per 4 quadrats.		No. of blocks.	Light intensity.	Plants per 4 quadrats.		No. of blocks.	Light intensity.	Plants per 4 quadrats.	
1	0.19	1		2	0.15-0.20	0.5		15	0.01-0.02	27.4		8	0.00-0.01	19.8	
1	0.23	0		8	0.20-0.25	26.3		10	0.02-0.03	15.0		25	0.01-0.02	22.5	
1	0.31	6		10	0.25-0.30	17.7		9	0.03-0.04	27.0		13	0.02-0.03	24.3	
8	0.35-0.40	14.9		13	0.30-0.35	37.5		14	0.04-0.05	29.5		0	0.03-0.04	—	
16	0.40-0.45	29.3		12	0.35-0.40	23.5		2	0.05-0.06	24.5		2	0.04-0.05	74.5	
15	0.45-0.50	24.2		3	0.40-0.45	23.0						1	0.05-0.06	49.0	
6	0.50-0.55	42.7		2	0.45-0.50	23.0						1	0.06-0.07	34.0	
2	0.55-0.60	27.3													
Mean light intensity	0.430				0.318				0.031				0.020		
Regression coefficient	15.8				16.0				20.3				71.0		
Significant level	$P < 0.01$				$P < 0.01$				Not significant				$P < 0.01$		
'Extinction point' (full daylight = 1)	0.15				0.03				—				< 0		

was zero, bluebells might still be expected. That is to say that plants can be found existing below their compensation points at this time of year, i.e. living on the reserves accumulated during the earlier high light phase.

In addition to considering each occasion separately, there is some advantage in calculating the regression of the average light intensity against density. As it has been stated before, the bluebell starts active growth in April and thus it is the mean light intensity subsequent to this date that is important. Since the curve of falling light intensity between March 30 and June is sigmoid, an

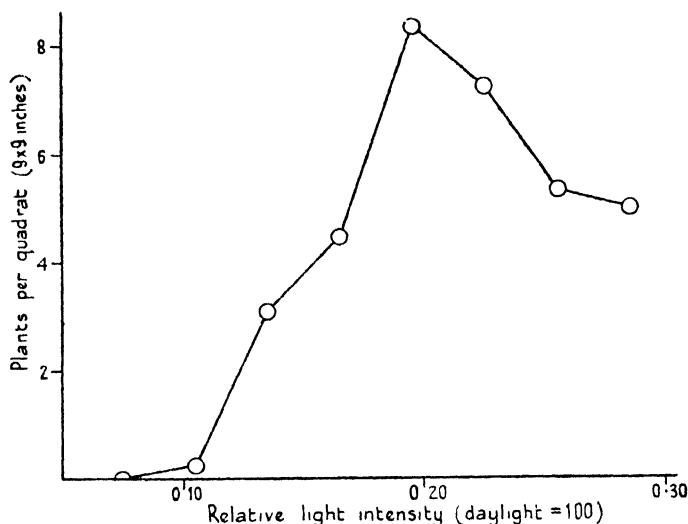


FIG. 6. Experiment II, Beech wood. The relationship between bluebell density and the mean degree of shading at 'ground' level, between March and June.

arithmetic mean of the light intensity on the four occasions in March, April, May and June ( $x_1, \dots, x_4$ ) is erroneous, and it was found empirically that the best simple approximation for the mean was obtained by calculating

$$\frac{x_1 + 2x_2 + 2x_3 + x_4}{6}$$

Employing this weighted mean for the spring light intensity ( $x$ ), the regression of the square root of plant density ( $y$ ) on light intensity is:

$$y = 28.5x - 0.93 \quad (\text{standard error of } b = 0.084).$$

By equating the density term to nought, the light value for the 'extinction point' proves to be 0.033 of daylight—a figure corresponding to the value calculated from the light data of the second occasion on April 24, see Table IV.

Finally, by dividing the mean seasonal light intensity for the 200 points into classes of equal intervals and determining the mean plant density within each class from the corresponding density data, the relationship between density and light intensity can be found. This is shown graphically in Fig. 6.

The shape of the curve would suggest that at the higher light intensities some factor other than light intensity may be operative in governing the distribution.

(iii) *The distribution under hollies in a beech-ash woodland.* The third site investigated was another plateau wood on the Berkshire Downs at Yattendon Common, about a mile distant from the beech wood of experiment II. In some parts of the wood both exotic deciduous and evergreen trees had been planted, but in the area chosen for the observations the predominant trees were large ashes and beeches with a few sycamores. The trees formed a high but somewhat open canopy, and beneath them scattered thinly were isolated hollies and an occasional yew. The ground flora was characterized by a dense carpet of bluebells, but it was very noticeable that beneath both the holly and yew trees bluebells were sparse or absent. More detailed examination showed that round the trunk of each holly no bluebells were present, and this zone extended to within a few feet of the spread of each holly. In the peripheral area there were bluebells, but the density was clearly less than outside away from the canopy of the individual hollies.

On general grounds it seemed that the sparseness of bluebells beneath the hollies might be due either to the deep shade beneath the canopy, i.e. a light factor, or to some other set of factors such as competition with the holly tree for moisture or nutrients. It was considered that the relative values of the light factor and the 'tree' factor could be determined by applying the statistical technique used for the larch-oak experiment, namely the fitting of a multiple regression with a pseudo-variate.

With this end in view, five hollies were chosen at some distance apart and three random transects, radial from the trunk, selected for each tree. Along each transect starting 2 ft. from the base of the tree 11 points were marked out at 2-ft. intervals—165 points in all. At every point the number of plants of *S. non-scripta* in a 9-in. square quadrat were counted and the light intensity relative to daylight measured on March 29, April 24, May 18, and June 26.

Observations were also made on the plants associated with *S. non-scripta* in the ground flora. Compared with the bluebell no other species was common and none was found beneath the hollies. Along five of the fifteen transects other species were absent, in a sixth there were only a few plants of *A. filix-mas*, and in two others *Mercurialis perennis* was occasionally present. In six of the remaining transects *M. perennis* (o) was associated with *Glechoma hederacea* (o), together with sycamore seedlings (o) in three instances, and *Rubus fruticosus* (o) and *G. hederacea* (r) in one instance each. Finally along one transect *R. fruticosus* (o), sycamore and ash seedlings (o), and *Viola* were present.

As in experiment II an average light intensity over the period of active growth of *S. non-scripta* has been calculated from the data of the four occasions

( $x_1, \dots, x_4$ ) by determining  $\frac{x_1 + 2x_2 + 2x_3 + x_4}{6}$  for each of the 165 points. The

resulting mean figures were divided into 9 equal-frequency classes and the

mean density of bluebells for the corresponding quadrat determined. The relationship between density and the mean seasonal light intensity is shown in Fig. 7. It is at once evident that there is a close relationship between light intensity and bluebell density.

In the statistical analysis of this relationship, three variates were considered:  $y$  the square root of the number of plants per quadrat,  $x$  the mean light intensity as a fraction of daylight, and a pseudo-variate ( $z$ ) to represent any possible

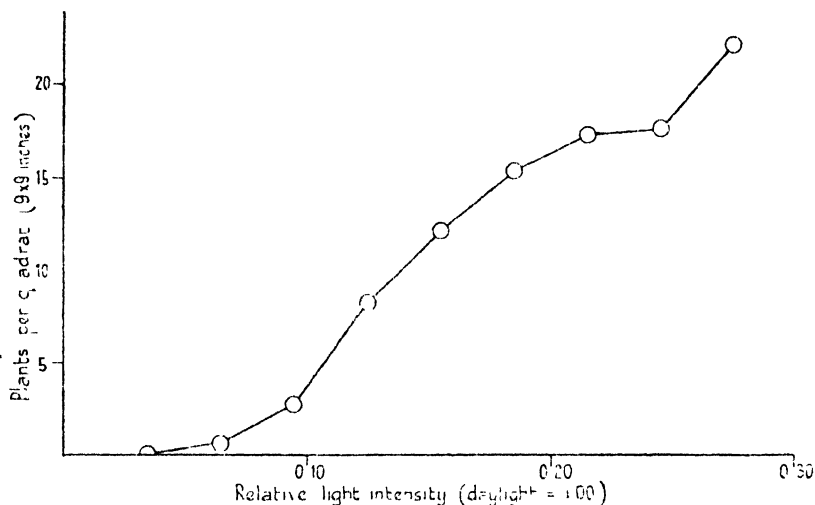


FIG. 7. Experiment III. Hollies in beech-ash wood. The relationship between bluebell density and the mean degree of shading at 'ground' level between March and June.

effect of the holly trees other than a light effect. For this purpose a value of 1 or 0 was assigned to each point whether it was without or within the canopy of the holly trees.

For statistical reasons previously given, the two points nearest the trunk on each transect were discarded since the value of the density variate ( $y$ ) was in every instance nought. With these omissions, an analysis of variance of density on the effects of individual holly trees and the distances of the sampling quadrats from the main trunk has been calculated (Table V). In the calculation the variance due to tree differences and its appropriate error were first found and subsequently the remaining variation within trees was analysed further.

It is evident that the largest part of the variation of density is due to the varying distance from the trunk. But this term has only 8 degrees of freedom, and in seeking to analyse this effect further by fitting a multiple regression on plant density and mean light intensity only 6 degrees of freedom would be left for error. Consequently in calculating the regressions the error ii term has been used, and this procedure has the advantage that positional effects are eliminated.



TABLE V

*Experiment III. Distribution of S. non-scripta under Hollies in a Beech-Ash Wood. Analysis of Variance of the Square Root of Plant Density on differences between Individual Trees and Distances from Main Trunk*

	Degrees of freedom.	Sums of squares.
Trees . . . . .	4	106.13
Error i . . . . .	10	25.34
Total i . . . . .	14	131.47
Distance from trunk . . . . .	8	174.28
Tree $\times$ distance . . . . .	32	35.22
Error ii . . . . .	80	60.18
Total . . . . .	134	401.15

TABLE VI

*Experiment III. Distribution of S. non-scripta under Hollies in a Beech-Ash Wood. Analysis of Variance of Regressions of the Square Root of Plant Density ( $y$ ) on Mean Light Intensity ( $x$ ) and a Factor for Presence within or without the Canopy of Holly Trees ( $z$ )*

	Degrees of freedom.	Sums of squares.	Variance.	$F$ .	$F$ required. 5 per cent.	1 per cent.	
Regr. on $x$ . . . . .	1	18.27	18.27	33.83	3.96	6.95	* *
Partial regr. on $z$ . . . . .	1	0.15	0.15	—	—	—	
Regr. on $z$ . . . . .	1	2.44	2.44	4.52	„	„	*
Partial regr. on $x$ . . . . .	1	15.98	15.98	29.59	„	„	* *
Multiple regr. on $x$ and $z$ . . . . .	2	18.42	9.21	17.06	—	4.88	* *
Remainder . . . . .	78	41.76	0.54	—	—	—	
Total . . . . .	80	60.18	—	—	—	—	

It is seen in Table VI that the multiple regression of the square root of density on light intensity and the tree factor is highly significant; this is equally true for the total and partial regressions of density on light intensity. The total regression on the tree factor is also significant. This relationship is to be expected, since there was a considerable reduction in light intensity within the canopy of the holly tree. But the partial regression of density on the tree factor is not significant. From this the tentative conclusion can be drawn that once the additional shading effect of the holly trees has been eliminated, the holly trees exert no other effect on the density of the bluebells.

The multiple regression relating the square root of plant density ( $y$ ) to the mean light intensity ( $x$ ) and the holly tree factor ( $z$ ) is given by

$$y = 21.3894x - 0.0004z - 0.149,$$

while the total regression of the square root of density on light intensity is

$$y = 21.389x - 1.072.$$

By substituting the value of nought for the square root of plant density in the second equation, the 'extinction point' can be determined and the value of 0.051 daylight is obtained for the light level at which bluebells are absent.

A multiple regression of the square root of density on the four separate measurements of light intensity has also been calculated. For this purpose the three points nearest the trunk on each transect have been discarded on account of the bias which they cause in the statistical calculations, due to the high proportion of quadrats containing no plants. The remaining eight quadrats on each transect were grouped together in adjacent pairs, and the corresponding pairs on the three radial transects of one tree were also grouped, giving 4 sets of figures per tree, and 20 sets in all. The object of this reduction of the data was to shorten the involved calculation necessary to the solution of five simultaneous equations. The following regression was obtained:

$$y = -8.4x_1 + 53.8x_2 - 16.7x_3 + 0.4x_4 - 1.68,$$

where  $y$  is the square root of the density in the six grouped quadrats, and  $x_1, \dots, x_4$  are the light intensities as fractions of daylight on the four occasions. That the regression is highly significant is shown by the analysis of variance set out in Table VII.

TABLE VII

*Experiment III. Distribution of S. non-scripta in a Beech-Ash Wood. Analysis of Variance due to the Multiple Regression of Plant Density on Successive Values of Light Intensity in March, April, May, and June*

	Degrees of freedom.	Sums of squares.	Variance.	F.
Multiple regr.	4	142.15	35.53	10.24 * *
Error . . . . .	15	52.04	3.47	—
Total . . . . .	19	194.19	—	—

To determine the significance of the partial regression coefficients,  $t$  tests were applied and gave values of 0.295, 1.142, 0.227, and 0.004 respectively. Since the value of  $t$  required at the  $P = 0.05$  level of significance is 2.131, none of the coefficients therefore is significant and only the partial regression for April ( $b_2$ ) even approaches significance.

Of the total regression coefficients all except that for June were highly significant; they are given in Table VIII together with a summary of all the relevant data. The extinction points for each occasion have also been determined by substituting nought for plant density in each regression, and these values are also given in Table VIII. As in experiment II, the values of the extinction point once the leaf canopy has expanded are lower than in the earlier high light phase.

## 2. Seasonal changes of light intensity in woodland.

There is one aspect of the light factor which has not yet been considered, namely the seasonal change in intensity on the floor of woodland. In both the beech wood experiment II and in the beech-ash wood (experiment III)

TABLE VIII  
*Experiment III. The Relationship between Density of S. non-scripta and Light Intensity on Four Occasions in a Beech-Ash Wood (Light Intensity expressed in Terms of Full Daylight)*

March 29				April 24				May 18				June 26			
No. of blocks.	Light intensity.	No. of plants per quadrat.	No. of blocks.	Light intensity.	No. of plants per quadrat.	No. of blocks.	Light intensity.	No. of blocks.	Light intensity.	No. of plants per quadrat.	No. of blocks.	Light intensity.	No. of plants per quadrat.	No. of blocks.	Light intensity.
12	0.10-0.13	3	12	0.10-0.13	3	6	0.02-0.04	6	0.02-0.04	0	30	0.01-0.02	47.6	30	0.01-0.02
6	0.13-0.16	47	6	0.13-0.16	8	24	0.04-0.06	24	0.04-0.06	22.5	42	0.02-0.03	84.4	42	0.02-0.03
6	0.16-0.19	29	12	0.16-0.19	38	12	0.06-0.08	12	0.06-0.08	55.5	18	0.03-0.04	51.3	18	0.03-0.04
18	0.19-0.22	49.3	6	0.19-0.22	66	36	0.08-0.10	36	0.08-0.10	96.2	24	0.04-0.05	80.3	24	0.04-0.05
12	0.22-0.25	78.6	18	0.22-0.25	72.7	24	0.10-0.12	24	0.10-0.12	89.8	6	0.05-0.06	128.0	6	0.05-0.06
12	0.25-0.28	105.5	24	0.25-0.28	79.1	12	0.12-0.14	12	0.12-0.14	115.0	—	—	—	—	—
12	0.28-0.31	51.0	12	0.28-0.31	112.5	6	0.14-0.16	6	0.14-0.16	64.0	—	—	—	—	—
42	0.31-0.34	104.9	30	0.31-0.34	103.6	—	—	—	—	—	—	—	—	—	—
Mean light intensity	0.251			0.244			0.089		0.089			0.030			0.030
Regression coefficient	33.0			38.7			72.0		72.0			99.7			99.7
Significance level	$P < 0.01$			$P < 0.01$			$P < 0.01$		$P < 0.01$			Not significant			Not significant
'Extinction point' (full daylight = 1)	0.013			0.041			$< 0$		$< 0$			—			—

the trend of these light changes can be evaluated from the light intensity data collected on the four successive occasions. From the measurements it is not only possible to calculate the general trend, but also to determine whether the trend differs according to the degree of shade cast by the overhead canopy.

For this purpose the 200 points of experiment II at which light observations were recorded have been divided into five classes of light intensity, on the basis of the degree of shade prevailing when the beeches were still dormant (March 29). In each class the light intensities on the three succeeding occasions have been expressed as a percentage of the initial intensity in March.

TABLE IX

*Experiment II. The Seasonal Changes in Light Intensity on the Floor of a Beech Wood following on Leaf Expansion of the Canopy in the Spring (Later Light Intensities are expressed as Percentages of the Initial March Value)*

Range of initial light intensity (daylight = 1).	Number of observations.	Light intensity.			
		March 29.	April 24.	May 18.	June 26.
0.10-0.20 . .	3	100	80.0	9.7	4.9
0.20-0.30 . .	7	100	75.4	9.4	3.5
0.30-0.40 . .	38	100	77.9	6.2	3.1
0.40-0.50 . .	125	100	75.0	7.5	4.4
0.50-0.60 . .	27	100	55.0	7.5	6.0

It is evident from the data of Table IX that in spite of the wide initial range in the degree of shading, the relative fall in the light values during leaf expansion is similar.

The data from experiment III in the beech-ash wood can be treated in the same way; the 165 initial values have been divided into four classes of light intensity. In the two lower classes most of the observational points lay within the canopy of the several holly trees, while the two remaining classes were only subject to the shade cast by the high canopy of beech, ash, and sycamore. It is not, therefore, surprising that the seasonal trend in light intensity differs between the lower and higher classes (vide Table X). The decrease in the shading effect between March and April at the lower light intensities is ascribed to the partial shedding of the holly leaves over this period.

TABLE X

*Experiment III. The Seasonal Changes in Light Intensity within and without the Canopy of Holly Trees under a higher Canopy of Mixed Deciduous Woodland (Later Light Intensities expressed as Percentages of the Initial March Value)*

Range of initial light intensity (daylight = 1).	Number of observations.	Light intensity.			
		March 29.	April 24.	May 18.	June 26.
Within holly trees:					
0.0-0.10 of daylight	29	100	110.5	34.3	13.7
0.10-0.20 " "	46	100	105.9	33.2	11.4
Without holly trees:					
0.20-0.30 . .	53	100	95.4	32.1	11.7
0.30-0.40 . .	37	100	94.2	36.6	13.7

The differences in the seasonal fall in light intensity between the two woodland communities can best be seen in Fig. 8, where for the sake of clarity the initial March light values in both experiments have been grouped into only two classes. It is evident that the trends show considerable divergence. In the beech wood the light intensity starts to fall earlier in the spring and reaches by mid-season much lower values than in the mixed woodland community.

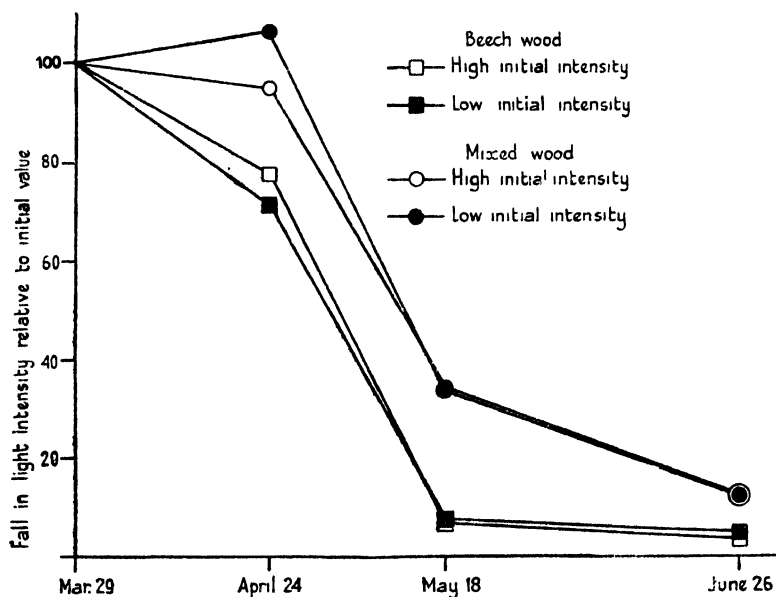


FIG. 8. Seasonal fall in light intensity at 'ground' level within a beech wood (experiment II) and a mixed wood of hollies, beech, and ash (experiment III). The results have been expressed relative to the values obtained in March prior to leaf expansion of the deciduous trees. For each wood a comparison is given for sites with a high or low initial degree of shading.

## DISCUSSION

In each of the woodland sites, by the design of the experimental procedure, coupled with the appropriate statistical treatment of the data, it has been possible not only to establish the primary importance of the light factor but also to assess the extent to which this factor is operative in each community. In the mixed deciduous wood (experiment III) it has been demonstrated that *three-quarters* of the variation in bluebell density can be accounted for solely by differences in light intensity during the period of active growth of the bluebell (Table VII). Moreover, by employing the technique of including a pseudo-variate in the calculating of the regressions of density and light intensity, it can be inferred that the low density beneath the holly tree is directly related to the deeper shade, and that other factors, such as soil moisture, slow rate of holly leaf decomposition, &c., are of little or no importance.

Similarly in the beech wood (experiment II) variations in the light intensity during the spring and early summer again account for half the total variation in density. Finally in experiment I, where Japanese larches predominated, a considerable proportion of the variability in bluebell numbers can be expressed in terms of shading, even though only a single series of light intensity measurements was made in early May.

Since in each of the communities a high correlation between density and shading has been established on observations based only on a *single* season, it can be concluded that changes in light intensity from season to season must have been followed closely by changes in density. In each of the woods there is little doubt that over a period of years the proportion of light reaching the ground flora will have gradually decreased. It is unlikely, however, that the yearly decrease would lead to an immediate alteration in density. Rather a lag phase is to be expected; the extent of the lag depending on the variation in the size and age of the individual plants, and the rate at which the degree of shading increases.

The existence of such a lag phase will not appreciably affect the assessment of the part played by light intensity in controlling the general distribution of the bluebell, but the lag must be taken into account when considering the light values for the 'extinction points'. By the fitting and extrapolation of the regressions of light intensity on density, the estimates of the minimal light requirement for survival or establishment have been made far more precise than those recorded by previous workers. Nevertheless, the 'extinction point' values of 0.11, 0.03, and 0.05 of daylight in experiments I-III, though they are based on regressions fitted to 345, 200, and 135 points respectively, are liable to some error.

In each of the communities the bluebell population varied from new seedlings to mature plants and other experiments have shown that the period of survival, once the light intensity has fallen below the compensation point, is dependent on plant size. Thus, where over a long-term period, as in these woods, the degree of shade is increasing, the larger plants will survive for more than one season below their 'compensation point'. In consequence the estimate of the 'extinction point' from the regression of light intensity on density is likely to be below the real value.

This source of error will be minimal where from year to year the general level of shade is falling slowly, and most where the fall is rapid. In this respect therefore the value of the 'extinction point' for experiment II can be taken as having the smallest error, since the density of the closed canopy of the large beeches is unlikely to have changed rapidly from season to season.

On the other hand, in experiment III the 'extinction point' value is probably too low since there is evidence that at least beneath the holly trees there was an appreciable fall in light intensity from season to season. Observations subsequent to 1939 showed that the periphery of the canopy of the individual holly trees was expanding. Thus with this growth the degree of shading

for sites beneath the holly trees, especially towards the edge, will change more rapidly than for sites under only the high canopy of the beeches and ashes. Under the evergreen hollies, particularly before leaf expansion of the overhead canopy, lateral light contributes largely to the total light intensity. Any extension therefore of the low-spreading branches of the holly trees will alter greatly the proportion of the lateral light.

Though the value of the 'extinction point' in experiment III is an underestimate, its value probably lies intermediate between the estimate of 0.03 in experiment II and that of 0.11 of experiment I. There is thus a considerable divergence in the figures for the three woodland communities and the question arises what are the factors contributing to this range. In experiments II and III it is not to be expected that there should be more than approximate agreement because it is clear from Fig. 8 that the trends in the seasonal fall of light intensity are considerably divergent. Again it is evident from the multiple regressions connecting density and light intensity that in both woods it is the high light phase in the early spring which contributes most to controlling the distribution. In this respect the average levels of light in late March and April are very different in the two woods, i.e. 0.44 and 0.32 of daylight in experiment II and 0.25 and 0.24 in experiment III. Thus in experiment II as far as the light factor in early spring is concerned the potential rate of growth is higher than in experiment III and thus may more than offset more advantageously the effects of the lower light phase later in the season.

In both experiments it is evident from Tables IV and VIII that the estimated 'extinction point' values vary greatly with the occasion on which the light observations were recorded. It may therefore be argued that in experiment I a very different value for the 'extinction point' might have been obtained if the single set of light observations had been taken earlier or later in the season, especially since there were both larch and oaks present. On the other hand, the larches largely predominated and on the basis of other experiments, to be discussed more fully in subsequent papers, the date of May 5 was chosen as representing (*a*) a point half-way through the period of active growth of the bluebell, and (*b*) a time when the average value of the light intensity of the larch wood was likely to be mid-way between the level before leaf emergence in early April and the level after full leaf expansion in June. When two years later in another part of the same larch wood the decrease in light intensity during the season was recorded, it was found that the light intensity fell from late March to a constant level in June and that at the end of April the value was 60 per cent. of the initial value before leaf emergence of the canopy, while the final level in June was 40 per cent. Again it is seen in Tables IV and VIII that the 'extinction point' values obtained from the late April light records are in closest agreement with the values calculated from the mean seasonal light intensities from March to June. On this evidence therefore the 'extinction point' for the larch wood is based on a fair estimate of the average light intensity during the active growth period of the bluebell. The greater value therefore under the larches can be attributed to the absence of a high

light phase in early spring, since the onset of active growth of the bluebell coincides with the 'breaking' of the larches in this district.

From the foregoing consideration of the conditions in the three communities, some general conclusions can be drawn as to when the light factor is likely to be operative in affecting the distribution of *S. non-scripta* in woodland. In deciduous woods the duration of the early high light phase and the light intensities ruling at the time would appear to be more important than the degree of shade cast after full expansion of the canopy. It follows that the light conditions will be less favourable to the establishment or increase of the bluebell in woods where the dominant deciduous trees expand their foliage early in the spring. It also follows that the light conditions existing in ever-green woods will be most inimical to the growth of *S. non-scripta*.

In the present experiments the range of light intensities during the high and low light phase varied considerably. In experiment II the highest initial light intensity recorded was 0.67 of daylight; moreover, 85 per cent. of the initial values were less than 0.5 of daylight. In experiment III an initial value of 0.4 daylight was not exceeded and only 22 per cent. of the 165 points reached over 0.3 of daylight. In both communities the maximum average intensities over the period March to June did not range over 0.3 of daylight. In woodland communities therefore where the initial shade is of the order of half daylight and the mean light intensity less than a third, it can be concluded that light will be an important environmental factor. On general evidence, this assumption is likely to be correct for *all* deciduous woodland where the canopy is *closed*, but may not be true where the canopy is *open*, for in these sites the light conditions may be very different.

In this connexion a study has been made of the seasonal changes in the light intensity of an open oak wood at Warfield, Berkshire, and also of a coppiced chestnut plantation at East Malling, Kent. In the oak wood, where *Q. robur* was dominant, the light intensity when the oaks were still bare was 0.84 of daylight (mean of 96 readings) and the final value in June after full leaf expansion was 0.33 of daylight. In the chestnut (*Castanea vulgaris*) wood, light intensity records were taken in two areas, one on the edge where the canopy was incomplete and the other in the centre with a closed canopy. The seasonal changes in light intensity are given in Fig. 9. The data demonstrate that the degree of shading at all times was less where the canopy was broken; they also show that the seasonal fall in light intensity differed between the two situations. Once leaf expansion was initiated, the light intensity at the edge no longer followed the same trend as in the centre. As a result, the average intensity between the end of March and mid-June was much lighter in the open area (0.65 daylight) than in the centre (0.28 daylight). Thus on the basis of experiments I-III, it can be assumed that within the closed area of the chestnut canopy the light factor exerted a controlling influence on the distribution of the bluebell.

Both in the open oak wood and on the edge of the chestnut coppice, the effect of shading cannot be assessed from the evidence of this investigation,



since both the initial and average light intensities were markedly higher than in the three closed woodland communities. On general grounds it would be expected that as the level of light intensity rose the effect on the growth and development of the bluebell would decrease. Other experiments where plants of *S. non-scripta* have been grown under a controlled range of shading have found this to be so. It must not, however, be inferred that the bluebell, in

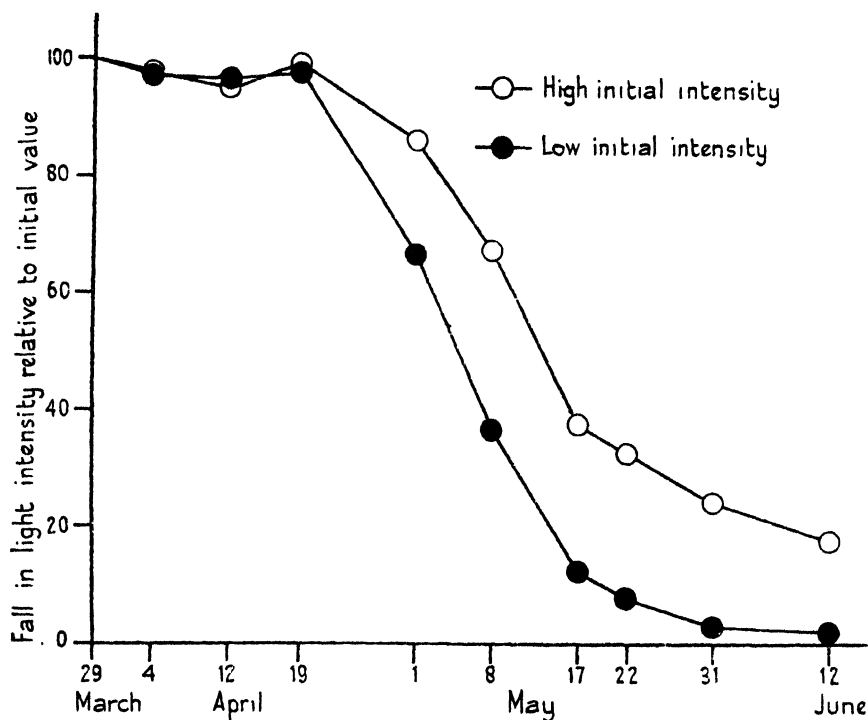


FIG. 9. Seasonal fall in light intensity within a coppiced Spanish chestnut plantation. The results have been expressed relative to the values obtained in March, when the chestnuts were dormant. A comparison is given for sites where the overhead leaf canopy was complete or broken.

spite of its prevalence in woodland, is an obligate shade plant. On the contrary the bluebell is as intolerant of shade as any plant. Nevertheless, open woodland is the most favourable habitat because of the absence of unfavourable factors such as trampling by animals, waterlogging, and a very shallow depth of soil.

#### SUMMARY

By the design of the experimental technique, coupled with the appropriate statistical treatment of the data, it has been possible to assess the relative importance of the light factor in governing the distribution of the bluebell, *S. non-scripta*, in three closed woodland communities. At some 165–365 points on either grid transects or radial transects from tree bases, counts of density

were made and light intensity measurements recorded. The degree of shading was estimated by comparing two matched photo-electric cells, screened with flashed, opal glass. Seasonal changes in light intensity were also investigated in other woods. The total number of light measurements involved exceeded 3,000.

In a larch wood, containing a few isolated oaks, the shade factor, based on a single series of observations in early May, was responsible for about one-third of the variations in the distribution of the bluebell. Similarly in a beech wood half of the fluctuations in bluebell density could be expressed in terms of the light intensities ruling between the end of March and the end of June. Finally, in a mixed deciduous woodland (beech, ash, and sycamore), with an understory of scattered hollies, the variations in shading over the spring and early summer periods accounted for three-quarters of the fluctuations in bluebell density.

By the technique of including a pseudo-variate in the statistical analysis it has been demonstrated that the absence or scarcity of bluebell beneath the holly trees in the mixed wood was solely due to the deeper shade. But evidence was obtained in the larch-oak wood that for a given light intensity conditions were less favourable to the bluebell underneath oaks than under larches. The combined effects of light intensity and this tree factor accounted for two-thirds of the variation in bluebell distribution.

In the beech and mixed deciduous woods multiple regressions of seasonal changes in light intensity on density were calculated. From these it is concluded that the distribution of the bluebell is governed more by the high light phase (period before and during leaf expansion of the canopy) than by the final low light phase after full leaf expansion.

From the extrapolation of the regression equations the minimal light requirements for survival or establishment of the bluebell can be estimated precisely. The values of the 'extinction points', i.e. the light level at which no bluebells will be found for the larch, mixed deciduous, and beech woods, were respectively 0.11, 0.05, and 0.03 of the mean daylight intensity between early April and mid-June.

In the beech wood the light intensity fell by mid-June to 4 per cent. of the initial value in March, and in the mixed deciduous wood the corresponding figure was 12 per cent. In both woods, with their *closed* canopy, the rate of fall was largely *independent* of the initial light intensity. But this was not so in a coppiced chestnut wood where the light changes in the spring and the ultimate depth of shade was dependent upon whether the canopy was open or closed; the relative reduction in light intensity between March and mid-June was to 18 and 2 per cent. of the initial values. The smallest fall in light intensity over the period from dormancy to full leaf expansion was recorded for an oak wood with a very open canopy, viz. 61 per cent.

It is concluded that the bluebell is intolerant of deep shade and that in most closed woodland communities light is the main environmental factor controlling distribution. It is also concluded that statistical methods and

techniques can make a valuable contribution to the solution of ecological field investigations.

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# Seasonal Changes in the Shoot apex of *Dryopteris aristata*

BY

HELEN L. FRAZER

(*Department of Cryptogamic Botany, University of Manchester*)

With Plate IX and seventeen Figures in the Text

## INTRODUCTION

THE importance of the shoot apex as the seat of growth and the region where the form and structure of the plant are determined has been adequately stressed (Schuepp, 1926; Sinnott, 1938; Wardlaw, 1945): in order to understand the formative influences which find expression during development apical growth must be investigated in all its aspects. The apex is a dynamic, physiological system continually changing under the influence of internal and external stimuli (Thoday, 1933), the form and structure observed at any one time being thus the product of these stimuli. This approach has perhaps been stressed chiefly in relation to angiosperms, but the pteridophyta also furnish suitable material for investigation and have not the added complication of secondary thickening.

One obvious expression of change is the rhythmical alternation of periods of activity and quiescence exhibited in the annual growth cycle of perennial species. It is of interest to inquire what changes can be detected at the apex coincident with this cycle. That such changes do occur in the apices of ferns has already been indicated (Wardlaw, 1944). In particular, investigations are required as to the distribution and utilization of metabolites, interactions between these in the apical region and the basipetal passage of stimuli from the apex, including the correlative inhibition of buds. The present investigation has been specifically concerned with the recording of variations in the morphology, histology, chemical composition, and water relations of the shoot apex of *Dryopteris aristata* at different times of the year.

## MATERIALS AND METHODS

For the investigations indicated above the species *Dryopteris aristata* was chosen because of the large size of the apical growing region and the comparative local abundance of the plant. Specimens were collected from the same wooded locality in Cheshire at fortnightly intervals during 1944 and 1945, care being taken to include only mature plants.

*Microscopical observations.* Where material was required for fixing, the apical region of the shoot was dissected in the field and fixed immediately to avoid the possibility of changes occurring prior to examination. For

histological observations formalin-acetic-alcohol and chrom-acetic fixatives were used, followed by staining with safranin and Delafield's haematoxylin, or chlor-azol-azurine (Armitage, 1943). The schedule of treatment was standardized so that differences in appearance would not be attributable to variations in treatment.

Observations on starch distribution were made on sections of fixed material stained with iodine, and the variations recorded by photography, care being taken to standardize each stage of the technique.

For other observations the living plants were kept overnight in a cool greenhouse and used on the day following collection. Cell acidity was estimated by the crude 'range indicator' method (Small, 1929). Freshly-cut hand sections were immersed in the various indicator solutions covering a range in pH of 3.4 to 6.2, and examined microscopically after one hour, without employing a cover-slip to avoid accumulation of carbon dioxide.

Distribution of unsaturated fats was studied by staining hand-sections of living material with osmic acid solution.

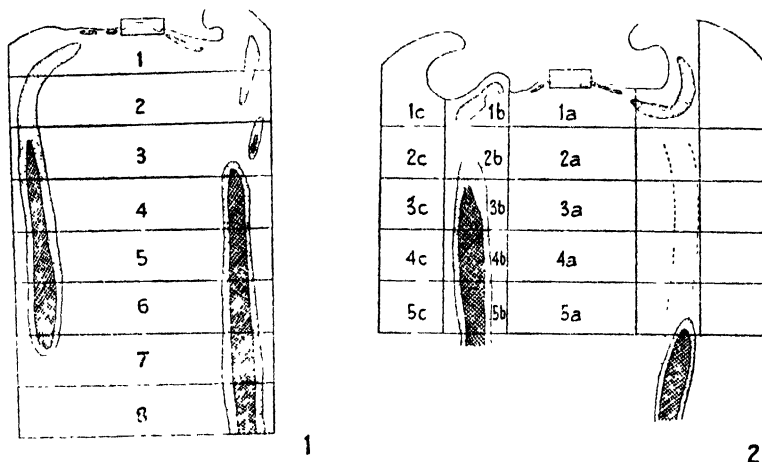
*Macroscopic observations.* Of necessity these relate to a larger region of the shoot. It was found convenient to divide the end of the shoot into zones 2 mm. deep, numbered 1 to 8 *from the apex backwards* (Text-figs. 1-2). Normally a cylinder of tissue 1 cm. in diameter was removed symmetrically round the apex with a cork-borer, and this was then divided transversely into 2 mm. disks. The morphological distribution of these and their relationship to the beginning of lignification in the xylem and to the area covered in the photomicrograph illustrations was relatively constant for plants of comparable size, and is indicated in Text-fig. 1. Some observations were also made by further subdividing the tissues; here three concentric cylinders of tissue of diameter  $1\frac{1}{2}$ , 1, and  $\frac{1}{2}$  cm. were removed from the shoot (Text-fig. 2) and divided into 2-mm. zones so that a small disk and two rings of tissue resulted for each zone. Data from these were rather less consistent indicating that excessive damage had been sustained by the tissues.

Dry-weight determinations were made after drying the tissues at 70° C. for 24 hours, and the result expressed as a percentage of the fresh weight. Each value is the average for three plants.

Osmotic pressure was estimated plasmolytically by immersing hand-sections in a graded series of sucrose solutions, and determining by subsequent microscopical inspection the strength of the solution in which plasmolysis just began. It was not, however, possible to count large numbers of cells because of the gummy nature of the cell contents. Suction pressure was determined by immersing weighed disks of tissue in graded sucrose solutions until equilibrium was reached, and re-weighing. After preliminary trials to estimate the approximate strength of the isotonic solution, fresh disks were placed in a solution of slightly higher concentration for 3 hours, weighed, and replaced in one slightly less concentrated for the same length of time. The order of immersion (stronger solution first) is important to avoid irreversible stretching of the plastic cell-walls in the growing region before both results are

obtained (Beck and Andrus, 1943). Total water uptake was estimated by weighing tissues after immersion for 24 hours in water, and was expressed as a percentage of the fresh weight.

*Statistical analysis of numerical data.* Results were presented as values for different zones at different dates of collecting throughout the year. Each figure represents the average for three plants examined on each occasion. By assuming that the values for each zone or for each date should be a replicate of others of the same kind, the data have been treated by analysis of variance.



TEXT-FIGS. 1-2. Diagrams to show the subdivisions of the apical region as used in the observations on dry-weight and water relations, and the relationship of these zones to the areas covered in the photomicrographs. ( $\times 33$ .)

Under 'remainder' (error) are included all differential responses. When this was done the differences due both to dates and to zones were found to be highly significant. With the data so far available it has not been possible to assess the precise significance of the difference between individual pairs of figures, but it is certainly safe to assume that the differences between extreme values both for zone and date variation are significant.

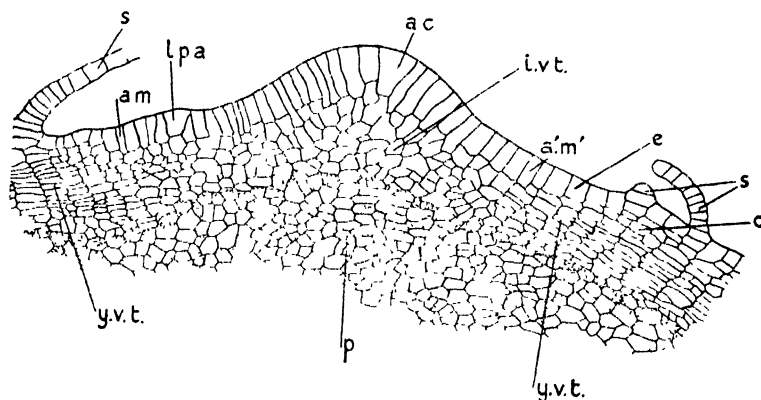
*Terminology.* Growth of the shoot in *Dryopteris* proceeds from a single large apical cell. The tissues formed by subsequent differentiation are shown in Text-fig. 3. For clarity the following terminology will be applied to them throughout the paper:

apical meristem: the layer of large superficial cells surrounding the apical cell and extending from *a.m.* to *a'.m'*. in Text-fig. 3;

incipient vascular tissue: vascular tissue in the initial phase of differentiation, situated immediately below the apical meristem, and derived from it by periclinal divisions (Wardlaw, 1944, p. 176);

young vascular tissue: the elongated meristematic cells of the vascular system situated between the incipient vascular tissue and the point where tracheids are first differentiated (see also Text-fig. 1).

Leaf primordia originate in the apical meristem, the initial cell of each arising by the oblique division of a single cell. The pith is differentiated from the basipetal side of the incipient vascular tissue (Text-fig. 3). At first the pith cells are meristematic, rounded, and non-vacuolated, but gradually intercellular spaces form and at a distance of 1.5 to 2 mm. behind the apical cell the cells elongate along the axis of the shoot. Epidermis and cortex are formed by division of cells on the basiscopic margin of the apical meristem.



TEXT-FIG. 3: Longitudinal section through the apex to illustrate the distribution of tissues. ( $\times 120$ .) *a.c.*, apical cell; *a.m.-a'.m'*., apical meristem; *e.*, epidermis; *c.*, cortex; *s.*, scale; *y.v.t.*, young vascular tissue; *p.*, pith; *l.p.a.*, apical cell of leaf primordium; *i.v.t.*, incipient vascular tissue.

## MORPHOLOGICAL OBSERVATIONS

### 1. External features

The duration of the various phases of the growth cycle as seen from external observation is as follows:

Mid-November to mid-April: plants dormant with crown exposed and previous season's leaves in various stages of decay.

Mid-April: young croziers starting to uncurl.

End of April: young croziers up to 15 in. high.

May: young croziers reaching full height.

Early June: pinnules expanded, sori small and green.

Mid-June: sori green, enlarging.

End of June: two-thirds of sori black in colour.

End of July: all spores shed.

Early September: some leaves beginning to discolour.

End of September: some leaves tending to droop.

End of October: many leaves still erect and green.<sup>1</sup>

Mid-November: leaves prostrate exposing crown of shoot.

During the period of dormancy the tissues of the shoot were drier and more

<sup>1</sup> The leaves of *D. aristata* tend to remain fresh and green much further into the autumn than do those of the related *D. filix-mas*.

brittle than during the growing season. In some plants, after the expansion of the first whorl of leaves, one or a few further young leaves may uncurl between May and August.

In the second season of the investigation a late frost in April damaged the tops of most leaves and spore-dispersal was delayed until early August.

A typical mature plant unrolls an average of 13–14 leaves each spring and has about 35 further leaf primordia between those which have opened and the apex.

## 2. Histology

The tissues of the meristem area react differently to stains at different times of the year. Four main types of staining occurred with safranin and haematoxylin:

- i. Cells in which the cell wall, cytoplasm, and nucleus remained deeply stained with safranin.
- ii. Cells in which the cytoplasm stained deeply with safranin, the nucleus and wall with haematoxylin.
- iii. Cells where the wall, contents, and especially the nucleus stained only with haematoxylin.
- iv. Cells in which only slight staining of the cell contents with both stains occurred and the wall stained with haematoxylin.

After fixing in formalin-acetic-alcohol, the apical cell and apical meristem stained deeply throughout the year (type i), the degree of staining being somewhat less in early June. The young vascular tissue was consistently of type iii. The greatest variation occurred in the incipient vascular tissue and in the pith. The incipient vascular tissue was clearly distinguished as type iv from the pith (type ii) during the summer from June till August (Pl. IX, Fig. 1). In spring and autumn, however, there was no distinction due to uniform retention of safranin by both (Pl. IX, Fig. 2). During the winter months the incipient vascular tissue was again visible because of deeper staining in the pith, but the contrast was not so marked as in summer (Pl. IX, Fig. 3).

Leaf primordia were also less deeply stained (type iv) during the period June till September. The small epidermal cells in the leaf axil tended to stain particularly deeply with safranin.

Cell divisions were observed from May until mid-October, i.e. beginning just before the incipient vascular tissue was clearly differentiated and ending after the staining difference ceased. In the leaf primordia cell divisions occurred when staining was minimal. The course of the cell divisions was as follows: before June periclinal divisions at the distal limit of the *young vascular tissue* and epidermis were most conspicuous. During June these divisions extended further towards the apical cell so that the lateral extent of the *incipient vascular tissue* was decreased. Towards the end of June and in July the cells of the apical meristem then multiplied by anticlinal divisions, and in August these newly formed units of the apical meristem widened again



to the normal dimensions. They also divided periclinally, the inner segments adding to the incipient vascular tissue. The extreme apex was thus the last region to resume growth.

#### CHEMICAL OBSERVATIONS

##### 1. *Hydrogen-ion concentration*

Priestley (1928) has suggested that cell divisions, e.g. cambial activity, occur in regions where there is a pH gradient. In such a region the proteins of some cells would be at the iso-electric point with least ability to swell in water, combine with salts, &c.—features which characterize meristematic cells. Selective absorption of stains has also been attributed to differences in the pH of the tissues, xylem being more acid than phloem. The indicator method with sections of tissues used here can only give a value which is difficult to relate to the condition of the intact cells. Nevertheless sufficiently consistent results were obtained to indicate that changes in pH which this method records are associated with the annual growth cycle. These seasonal changes were restricted to the apical meristem, incipient vascular tissue and pith (Text-figs. 4, 5). The *incipient vascular tissue* became less acid during the period of active growth in summer, the change being roughly coincident with the time when this tissue was most clearly differentiated in stained sections. The *apical meristem* became less acid during winter. This change was not associated with a staining difference in specimens fixed in formalin-acetic-alcohol, but after chrom-acetic fixative there was a lesser degree of staining in the cells with reduced acidity. The *pith* was most acid during spring and autumn.

##### 2. *Distribution of unsaturated fats*

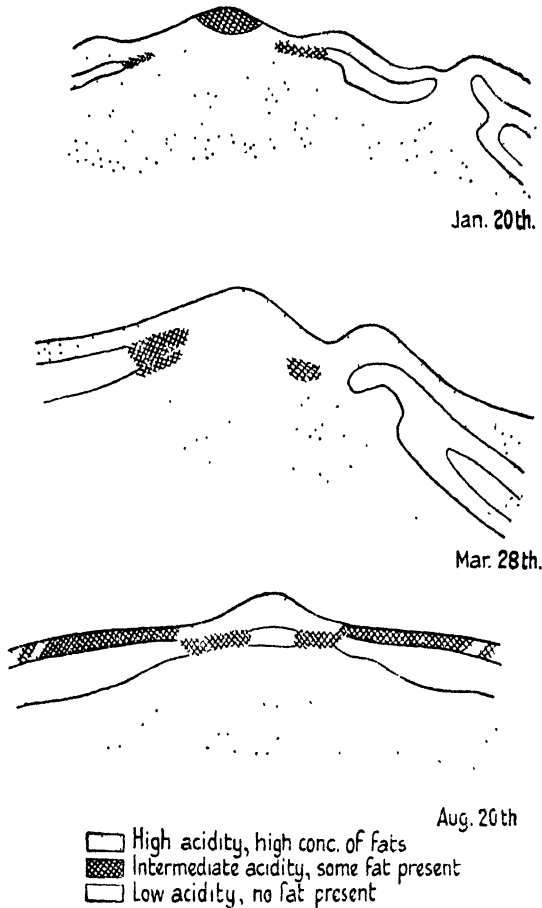
This was closely associated with the variations in acidity. No fat was present in the young vascular tissue which was the least acid region, only a slight reaction was obtained in regions of intermediate acidity, the highest concentration occurred in the most acid tissues.

##### 3. *Distribution of starch*

Starch is the most obvious metabolite in the fern shoot. Its great abundance indicates the efficiency of the photosynthetic system. Changes in starch distribution and density will, to some extent, be an index of growth activity.

Staining with iodine yielded the results shown in Pl. IX, Figs. 4 and 5, and Text-figs. 6–13. During the winter, every cell in the growing region was densely packed with starch grains which tended to accumulate round the nucleus. The deposition was slightly less in the young vascular tissue. In late April and in May starch density began to decrease, especially in the incipient vascular tissue and leaf primordia, reached a minimum in June and July, and then increased again from August onwards. The period of decrease in starch density corresponded to the time when the current year's leaves were uncurling. The period of minimum density was at the time when the spores were ripening and being shed. There was no time of the year, however, when

the apical cell and apical meristem were found to be free from numerous starch grains (Pl. I, Fig. 5): in the leaf primordia also, starch grains were constantly observed in the apical cell and large epidermal cells even during July when least starch was present. The starch density was greater in the outer

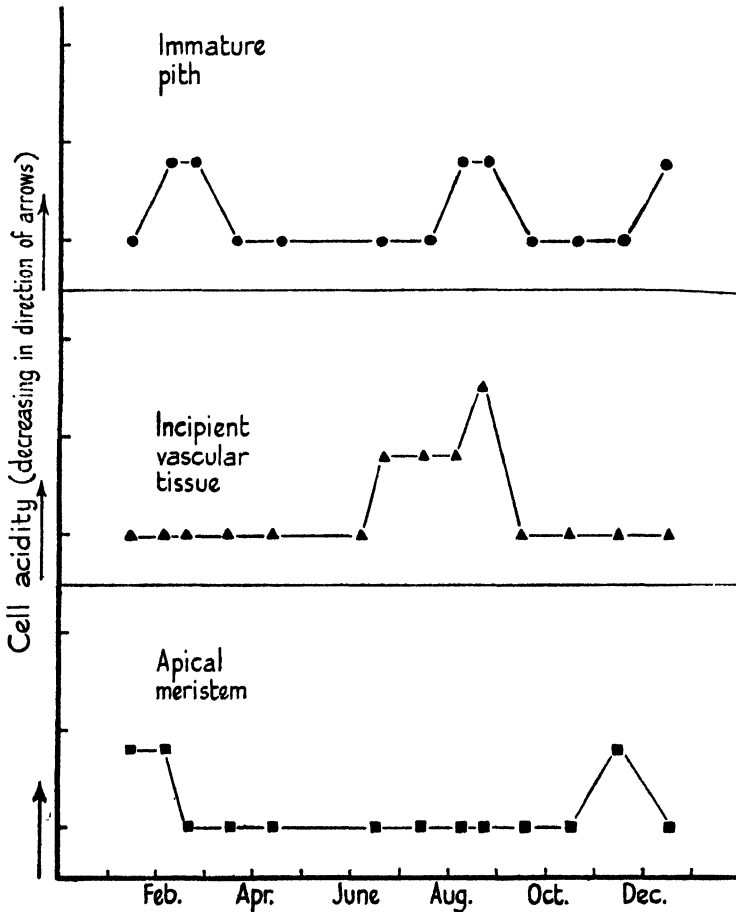


TEXT-FIG. 4: Diagram showing the pH and fat content (by tissue test) at different times of year.

side than in the axil of the primordia, except in winter, when starch was dense throughout (Text-figs. 11-13). There was never evident a clear line of separation in the growing region between tissues with and without starch such as occurred in the distribution of pH and fat. It may thus be concluded that carbohydrate supply does not at any time limit the growth of the shoot.

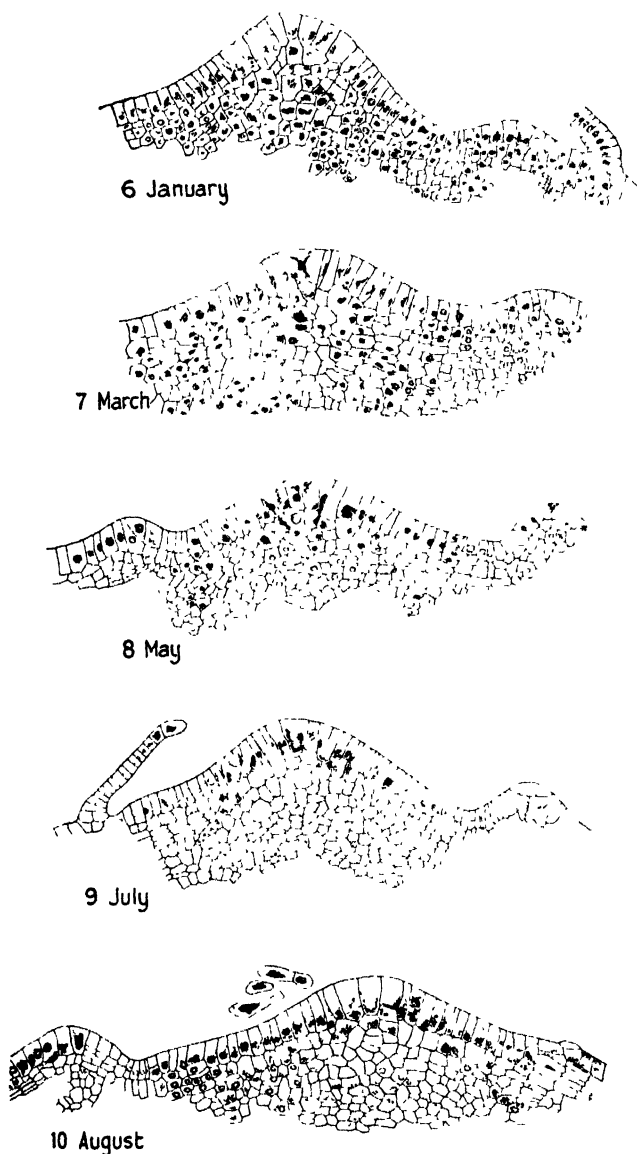
Since a large proportion of the dry material of the fern tissues consists of starch, changes in dry weight are probably related to variations in its distribution. Dry-weight measurements covering the parts of the shoot up to 16 mm.

from the apex (see methods) gave results closely comparable with the photographic records. There was a rapid decrease in dry weight from April till June, and then a more gradual increase again. When the tissues were separated into pith, vascular area and cortex (Text-fig. 14), closely comparable



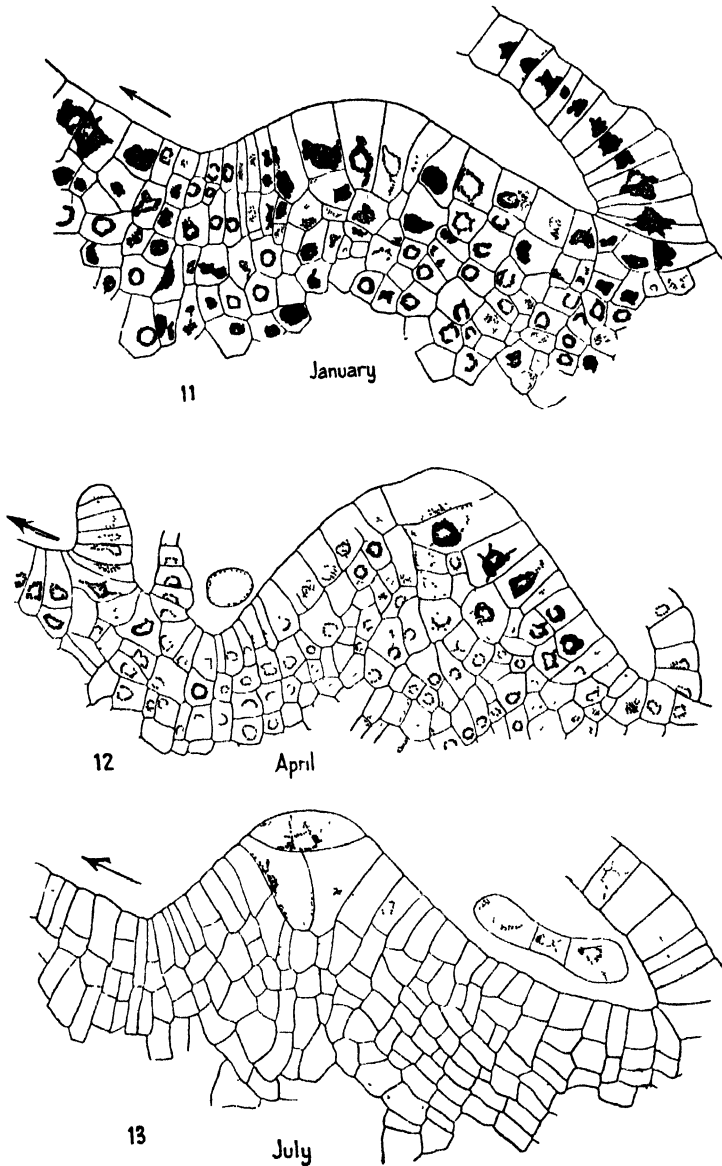
TEXT-FIG. 5: Graph showing seasonal variation in the pH (by tissue test) of the apical meristem, the incipient vascular tissue, and the pith.

changes in dry weight were observed throughout the year in each of these three regions. In zone 1 the dry weight was practically equal in pith and cortex, but from zone 2 backwards, the value for the pith was less than that of the vascular area or cortex. The differences between maximum and minimum dry weight were fairly constant in all zones, so that starch is used in equal quantity from all the regions investigated. The dry weight of the region of cell vacuolation was slightly less than that of the meristem itself: beyond the region of vacuolation the dry weight increased basipetally at all times of the year.



TEXT-FIGS. 6-10: Longitudinal sections through the apices of plants showing the starch distribution at different times of year. Fig. 6, January; Fig. 7, March; Fig. 8, May; Fig. 9, July; Fig. 10, August. ( $\times 52$ .)

A comparison of the dry-weight variation with seasonal temperature changes (Text-fig. 14) shows that the period of most rapid starch decrease (March-May) occurred when there was a rapid rise in temperature. Both the dry weight and temperature values were more steady from June till September.

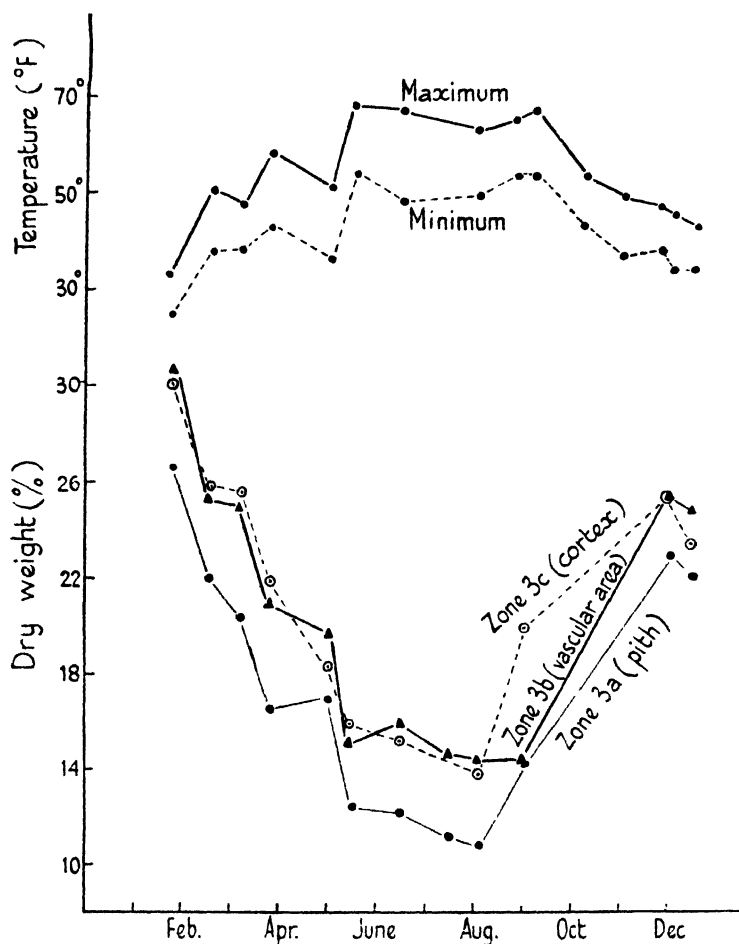


TEXT-FIGS. 11-13: Illustrations of starch distribution in leaf primordia at different times of year. Fig. 11, January; Fig. 12, April; Fig. 13, July. ( $\times 170$ .) The arrows point towards the apex.

#### 4. Distribution of nitrogen

Nitrogen is required at the apex for protein synthesis. Variations in its distribution and transport to the apex may affect meristematic activity to a greater extent than carbohydrate changes. Preliminary determinations have indicated that there was a decrease in the total nitrogen present in the terminal

16 mm. of the shoot during the early summer but further details are not yet available. This decrease is coincident with the decrease in dry weight over the same period. No separate values for protein and soluble nitrogen have yet been obtained. Wetzel (1938) also found that the total nitrogen in the



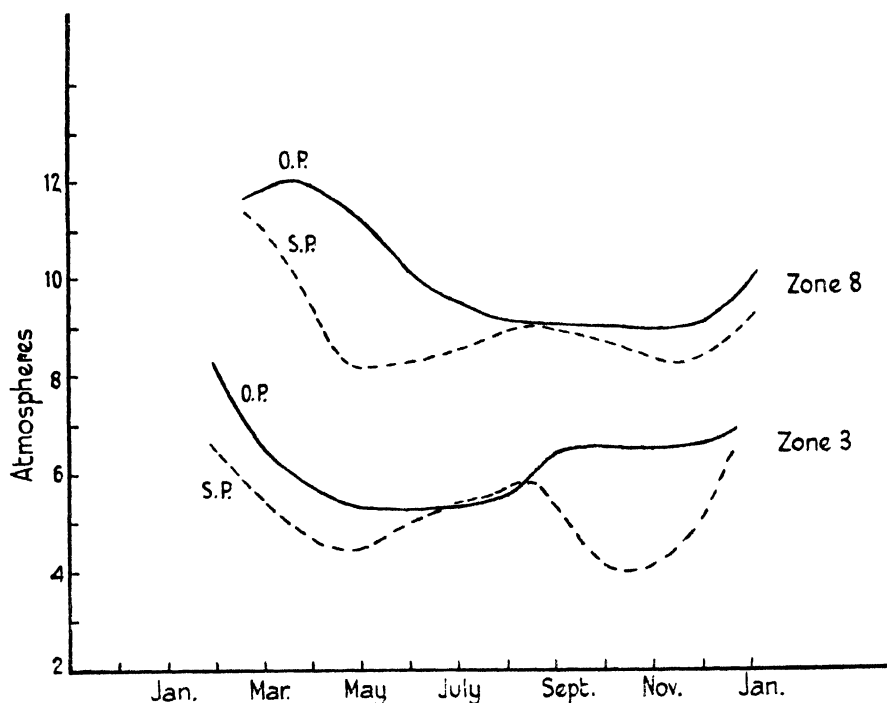
TEXT-FIG. 14: Graphs showing seasonal variation in dry weight of the pith, vascular region, and cortex in zone 3 (i.e. 4-6 mm. behind the apex). Above are shown the variations in the average maximum and minimum temperatures during the week before each date of collecting.

rhizome of *Dryopteris filix-mas* (as a percentage of the fresh weight) fell rapidly from April to August and then rose gradually till October when the measurements ceased. He states that the protein nitrogen remained fairly constant, but does not give any details of the part of the rhizome which was analysed.

#### OBSERVATIONS ON WATER RELATIONS

Cell vacuolation and elongation will occur only if there is intake of water by the cells and if the cell walls are in a plastic condition. This intake of water

is, in part at least, an osmotic phenomenon, and the experiments described in this section deal with variations in the osmotic and suction pressures of the tissues at different times of year. The transport of metabolites to the apex may also in part be affected by the availability of water.



TEXT-FIG. 15: Smoothed graphs showing seasonal changes in suction pressure (S.P.) and osmotic pressure (O.P.) of zones 3 and 8 (i.e. 4–6 mm. and 14–16 mm. behind the apex).

### 1. Osmotic pressure of the cell sap

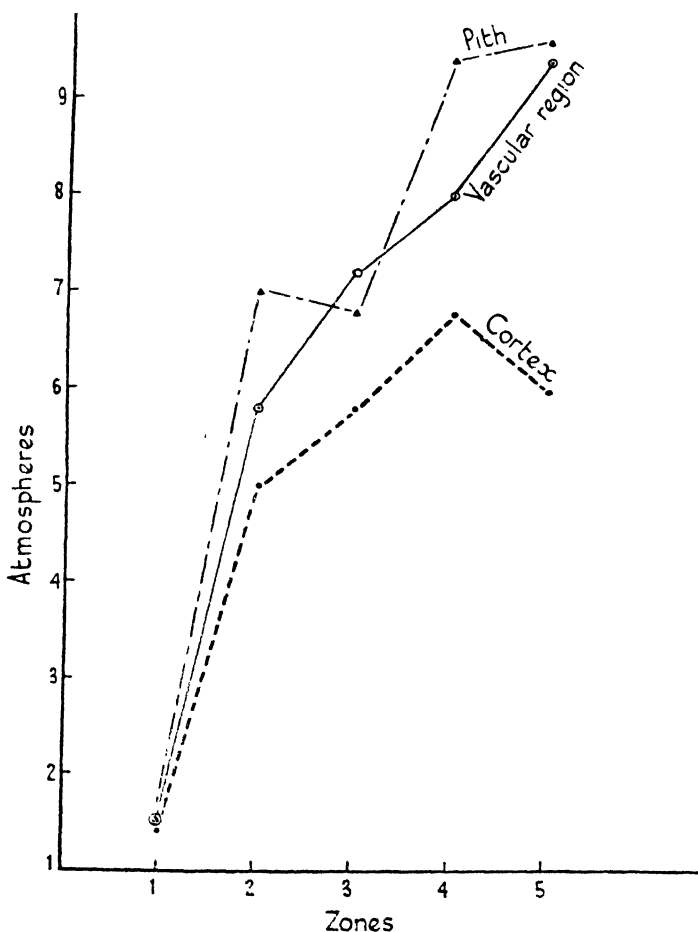
Differences in osmotic pressure due to different dates of collecting are significant. The osmotic pressure was lower in summer than in winter (Text-fig. 15). The incidence of the summer decrease was basipetal, zones 2 to 5 reacting earlier in the season than zones 6 to 8. Zone 1 cannot strictly be compared as the majority of its cells are non-vacuolated. There was also a significant basipetal increase in the osmotic pressure of the parenchyma at all times of year. The osmotic pressure of the cortical parenchyma was lower than that of the pith throughout.

### 2. Suction pressure

As with osmotic pressure, there was a significant basipetal increase in suction pressure at all times of the year. Variations due to seasonal changes were also significant. Spring and autumn were periods when the suction pressure was low, with a rise in summer (August) and in winter (Text-fig. 15).

Suction-pressure measurements carried out on smaller subdivisions of the

shoot (see Text-fig. 2) showed that the suction pressure of the pith was always higher than that of the cortex, with the values for the vascular area somewhere between in winter, but approximating to those of the pith in summer (Text-fig. 16).



TEXT-FIG. 16: Graph showing the suction pressure of pith, vascular area, and cortex at one date of collecting (March) for zone 7 (i.e. 12–14 mm. behind the apex).

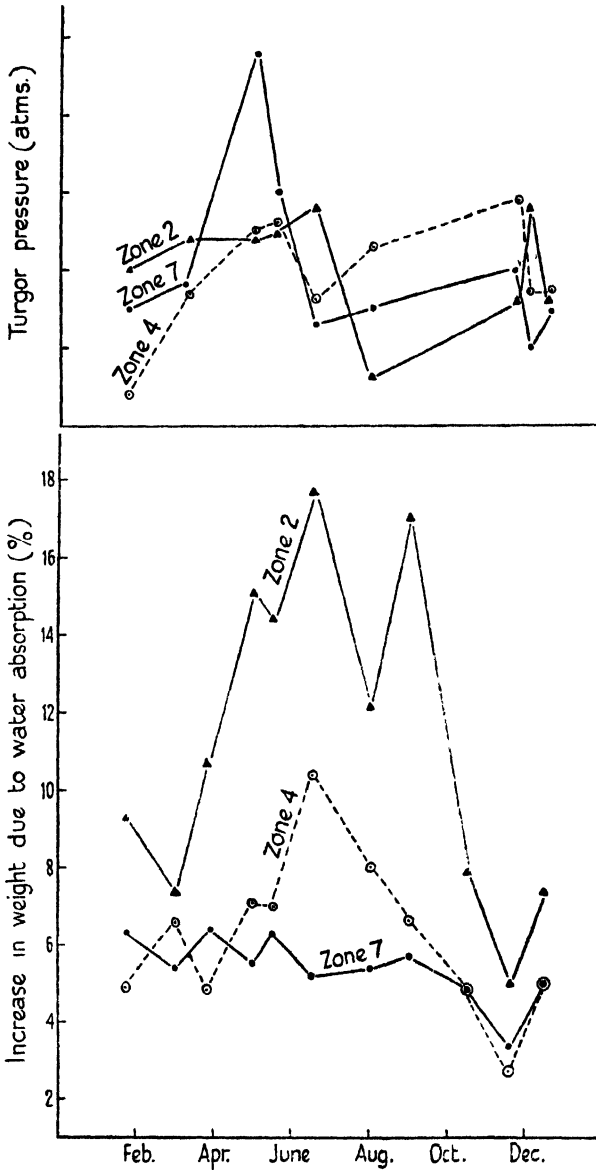
### 3. Turgor pressure

The difference between osmotic and suction pressure gives the turgor pressure (equal in magnitude to wall pressure). For all zones this was found to be low in summer (during June and July) and in winter, high in spring (March–May), and somewhat increased in late autumn (Text-fig. 17).

### 4. Maximum water uptake

The differences in water uptake due to date of sampling and to the position of the tissues examined are both significant. As is to be expected the tissues





TEXT-FIG. 17: Graph showing seasonal changes in water absorption (lower figures) and turgor pressure (upper figures) of zones 2, 4, and 7 (i.e. 2-4, 6-8, and 12-14 mm. behind the apex).

with greatest capacity to absorb water were in zones 2 and 3 at all times of the year, and the water absorption then decreased basipetally from zone 3 to zone 8. Zones 1 to 3 were also the regions which showed the greatest seasonal variation. There was a very marked increase in water-uptake capacity in these zones during the growing months, while in the fully mature and senescent

tissues farther back the difference between summer and winter absorption was practically eliminated (Text-fig. 17).

### 5. *Transport of materials to the apex*

This was investigated by observing the movement of dyes along the vascular tissue when plants were placed with the cut end of the stem stele in dye solution for several days. Basic dyes scarcely penetrated beyond the first layer of cells, but acid dyes (acid fuchsin, patent blue) readily travelled along the xylem: irrespective, however, of whether the plants were actively growing or dormant, penetration of the dyes in the direction of the apex always stopped at the youngest differentiated tracheid. There was no penetration of the dye into the surrounding parenchyma in the shoot itself, although in plants where the current year's leaves were uncurling there was some passage of acid fuchsin from the vascular tissue into the parenchyma at the tips of the leaves, especially in the region of greatest curvature near the top. Specimens were also prepared in which all the leaves were removed and the shoot planted in fibre until a number of further leaf primordia had formed, thus assuring that the apex was actively growing. When freshly cut bases of these were placed in dye solutions there was still no penetration of dye beyond the last formed tracheides into the young vascular tissue. Experiments in which scales and leaf primordia were removed from the apex and a small glass ring then inserted round the apex showed that considerable growth and formation of fresh leaf primordia took place, although the glass ring effectively severed the young vascular tissue. This confirms and strengthens Wardlaw's observations (1945*a*) that transport to the apex is independent of the young vascular tissue.

## DISCUSSION

*Morphological, histological, and chemical data.* Although the uncurling of leaves and a decrease in stored starch (in mature plants) starts in April, active growth by cell divisions in the meristem is not resumed till May. This resumption of growth in the apex is closely followed by changes in the incipient vascular tissue which are expressed as an altered capacity to retain stains in fixed material, a lessening of the acidity of the cells, and a decrease in the fat content. Kemp (1943) found that the zonal pattern in the apical region of *Torreya* was reduced to a minimum during dormancy and was at a maximum during the formation of appendages.

Other published data on seasonal variation in pH indicate that the H-ion concentration does vary in the same tissue at different times of year, but that there is considerable diversity of reaction from species to species. Changes in pH will affect protein swelling and therefore may be expected to affect the rate of penetration of solutes through the cytoplasm. Whether the association between pH changes and fat content indicates that these are inter-related, or is purely coincidental is not yet known.

Depletion of carbohydrate may be referred to an increase in respiration and new wall formation during growth. Wetzel (1938) reports Johansson's work

(1923 and 1926) on respiration in ferns where it was shown that an increase of temperature increased the respiration rate between 10° and 48° C. and that young leaves respired more rapidly than older ones in bracken. The persistence of starch in the apical meristem and the relative infrequency with which these cells divide indicates a slower rate of growth and probably a different kind of metabolism. The greater density of starch on the abaxial side of the leaf primordium indicates that more nutrient is supplied to that side. The cells are larger and growth is more rapid so that the primordium becomes characteristically incurved. The absence of any clear distinction between regions with and without starch at the junction between pith and incipient vascular tissue suggests that there is a continuous gradual upward diffusion of carbohydrate as growth proceeds.

The marked staining differences shown by the incipient vascular tissue in summer support Wardlaw's hypothesis (1944) that whenever an apex is in an active state of growth this is reflected by the origin of vascular tissue immediately behind it. There is, however, also the mid-winter differentiation in staining in this region (Pl. IX). It may be that in the non-growing *Dryopteris* apex during winter biochemical changes are still occurring which influence the tissues below, but that the visible expression of this is masked at certain times of year.

*Osmotic relations.* For purposes of discussion, the relevant data are as follows:

1. Turgor pressure is high in spring at the time when dry weight is decreasing, and capacity to take in water in zones 1-4 increasing.
2. Turgor pressure is low in summer when capacity to take in water is very high in zones 1-4, but unaltered in maturer tissues.
3. Osmotic pressure decreases in spring when turgor pressure is high, and remains low in summer when turgor pressure is low.
4. Turgor pressure increases somewhat in late autumn when dry weight has increased and osmotic pressure is increasing.
5. Osmotic and suction pressures are always lower in cortex than pith, and dry weight of cortex is higher than that of pith except in the terminal 2 mm. where they are about equal.

These data indicate that the increased capacity for water-intake in spring is not solely the result of the absence of complete turgor. Since the younger cells (in zones 2-3) are able to absorb an increasing amount of water at the time of year when they are most turgid (Text-fig. 17), the cell walls must be more plastic at this time in order to accommodate the absorbed water. In the more mature tissues (zone 4 backwards) there is no such increase in water-intake capacity in spring; presumably here the walls are no longer plastic. A basipetal decrease in cell-wall plasticity is in fact demonstrated. There would thus appear to be in April an increase in the factors responsible for cell elongation at a time just before growth by cell divisions is resumed at the apex in May; this increase shows reduction basipetally.

Increase in cell-wall plasticity has been attributed to auxin action (Heyn, 1940). Also significant is the constant lower suction pressure and osmotic pressure in the cortex than in the pith, and the higher dry weight of the cortex except at the extreme distal end of the shoot. Together these suggest that vacuolation does not proceed as far in the cortex as in the pith. Wardlaw (1945*b*) found that the ratio, sectional area of cortex—sectional area of pith, was very large in the immediate neighbourhood of the meristem but decreased rapidly with distance from the meristem. This increase in dimensions of the pith may largely be due to the higher osmotic forces of the pith cells. Near the apex, and to a lesser extent in the mature dictyostele (Wardlaw, 1945*b*), the external surface of the vascular tissue is greater than the internal, so that on a basis of simple diffusion of water or metabolites from the stele the cortex should receive more than the pith. The available evidence has indicated, however, that transport of metabolites in the meristem area is independent of the vascular system.

#### SUMMARY

1. Histological changes in the shoot apex of *Dryopteris aristata* have been studied (in double-stained sections) at frequent intervals during the annual growth cycle. The initial differentiation of vascular tissue is most clearly seen in summer when the shoot is actively growing. In winter the incipient vascular tissue is somewhat differentiated by staining, but in spring and autumn there is no visible differentiation between incipient vascular tissue and immature pith. The summer differences in staining occurred within the period when active growth by cell division was taking place in the apical region.

2. Growth begins in spring with the uncurling of the lowermost series of leaves. The extreme apex is the last region to resume growth.

3. Physiological changes investigated in conjunction with histological changes have included the seasonal variations in the pH (as measured by tissue slices in indicators) and in the fat-content, which occur in the apical meristem and incipient vascular tissue; the apical meristem by this method shows a higher acidity in summer than in winter, while the incipient vascular tissue is less acid in summer than in winter. Fat is absent from the least acid tissue.

4. The density of starch deposition and the dry-weight decrease during the period when the leaves are uncurling; they are minimal when the spores are being shed in July, and increase again in late summer and autumn. Starch remains abundant throughout the year in the apical meristem and is more dense in the abaxial side of leaf primordia than in the axil. The acropetal decrease in starch deposition from pith to incipient vascular tissue in summer is gradual, without any clear distinction between tissues with and without starch grains.

5. Measurements of suction pressure, osmotic pressure, and water-uptake capacity show that there is a basipetal decrease in cell-wall plasticity in summer. Capacity for water-uptake is greatly enhanced in summer in the

regions of cell elongation but not in the mature tissues. The suction and osmotic pressures of the pith are higher than those of the cortex. The bearing of these data on the relative distribution and development of the several tissue systems is discussed.

6. Transport of metabolites to the apex appears to be independent of the young vascular tissue.

The author wishes to express her gratitude to Professor C. W. Wardlaw for his continued advice, and to thank Mr. E. Hamson for permission to consult local weather reports, and Dr. L. G. G. Warne for advice on the statistical treatment of numerical data.

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#### EXPLANATION OF PLATE IX

Illustrating the paper by Dr. H. L. Frazer on Seasonal Changes in the Shoot Apex of *Dryopteris aristata*

All figures are from untouched photographs.

Figs. 1-3. Seasonal differences in staining of the apical region as seen in longitudinal sections fixed in formalin-acetic-alcohol. ( $\times 58$ .) Fig. 1, July; Fig. 2, October; Fig. 3, December.

Figs. 4-5. Seasonal differences in starch content as shown by sections of fixed apices stained with iodine. ( $\times 58$ .) Fig. 4, early April, at onset of growing season; Fig. 5, July, at height of growing season.



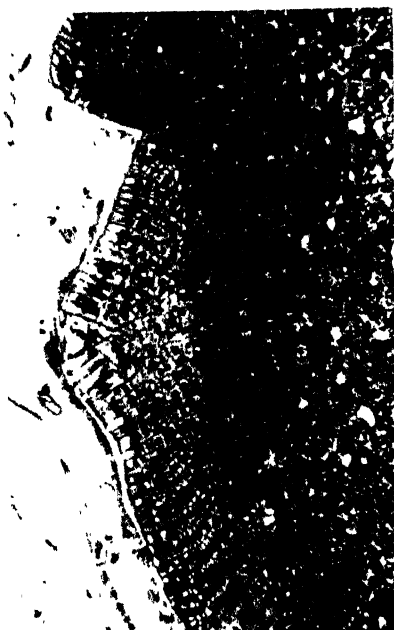
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FRAZER — DRYOPTERIS SHOOT APEX.



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